protonated alcohols,[15,16] with ammonia to yield protonated amines,[14] with HCN to yield protonated isocyanides,[17] and with CO to yield acylium ions.[18] To examine whether reactions 5 and 6 behave similarly, we calculate $\Delta H°_f$ of the products of reactions 5 and 6 as 42 and 35 kcal mol^{-1}, respectively. (Based on PA(NH_3) = 202.3 kcal mol^{-1}. Using PA(NH_3) ≈ 207 kcal mol^{-1}, we would obtain $\Delta H_f°$ = 37 and 30 kcal mol^{-1}, respectively.) Comparing with the heats of formation of $GlyH^+$ and $AlaH^+$ as given above, we find that the products of the association reactions are not identical with $GlyH^+$ and $AlaH^+$. This is in agreement with the conclusion we made on the basis of $\Delta S°$ of the association reactions. Furthermore, we make the unexpected observation that the products of reactions 5 and 6, which are thus assumed to be hydrogen-bonded cluster ions, are more stable by 11 and 8 kcal mol^{-1}, respectively, than their covalently bonded isomers $GlyH^+$ and $AlaH^+$. This intuitively unexpected result is rationalized on the basis that the heats of formation of the neutral species ($CH_3NH_2 + CO_2$) and $CH_2(NH_2)COOH$ are similar (−5.5 − 94.4 = −99.9 and −104 kcal mol^{-1}), respectively. However, the electron-withdrawing effect of -COOH in the amino acid destabilizes the charge on $CH_3(NH_3^+)COOH$, as we found earlier; in contrast, the ion-neutral electrostatic and hydrogen-bonding forces stabilize the charge in $CH_3NH_3^+ \cdot CO_2$. The greater stability of the cluster ion thus results from the opposite effects of intramolecular charge destabilization by -COOH in the covalently bonded structure as opposed to the intermolecular charge stabilization by CO_2 in the cluster ion.

In addition to proton affinity measurements, the determination of N_A by the present method makes it possible to study quantitatively other ion–molecule processes, such as association. For example, in the course of the present work we also observed the equilibrium reactions involved in the formation of the proton-bound dimers $(Gly)_2H^+$ and $(Pro)_2H^+$. Quantitative measurements of the equilibria yielded the values $\Delta H°$ = −31 ± 2 and −29 ± 2 kcal mol^{-1}, respectively, and $\Delta S°$ = −33 ± 5 and −32 ± 5 cal mol^{-1} K^{-1}, respectively. The $\Delta H°$ value for $(Pro)_2H^+$ is more negative than a previous result of 20 kcal mol^{-1}, which was obtained by a continuous ionization technique and a different sample introduction procedure.[19] The time-resolved result obtained here is more reliable. The enthalpies and entropies of association for the amino acids are more negative than those observed in the formation of protonated amine dimers,[20] which are generally in the range $\Delta H°$ = −21 to −25 kcal mol^{-1} and $\Delta S°$ = −24 to −27 cal mol^{-1} K^{-1}. The enhanced bonding and constrained geometry which are reflected in the thermodynamic values for the amino acids may be indicative of multiple hydrogen bonding in the protonated dimers of these polyfunctional molecules.

Acknowledgment. This work was supported by a grant from the National Science Foundation.

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(21) Recent values of $\Delta H°_f(t\text{-}C_4H_9\cdot)$ = 12 kcal mol^{-1} (W. Tsang, *Int. J. Chem. Kinet.*, **10**, 821 (1978)), combined with IP($t\text{-}C_4H_9\cdot$) = 151.7 kcal mol^{-1} (J. Dyke et al., quoted by F. A. Houle and J. L. Beauchamp, *J. Am. Chem. Soc.*, **100**, 3290 (1978), footnote 44) and $\Delta H°_f(H^+)$ = 365.7 kcal mol^{-1} can be used to obtain PA($i\text{-}C_4H_8$) = 198.0 kcal mol^{-1}. This value, combined with PA(NH_3) − PA($i\text{-}C_4H_8$) = 8.6 kcal mol^{-1} (ref 2), yields PA(NH_3) = 206.6 kcal mol^{-1}. This is higher than the value of PA(NH_3) = 202.3 kcal mol^{-1} which was used in ref 3 and throughout the present paper. Using the more recent reference value, all proton affinities will be higher, and all ion heats of formation will be lower by 4.3 kcal mol^{-1} than the values quoted in the paper. Note, however, that all the discussion in this work is based on relative proton affinities of amines and amino acids, and the conclusions are qualitatively and quantitatively unaffected by the absolute PA values.

Tetracyclines. 9. Total Synthesis of *dl*-Terramycin

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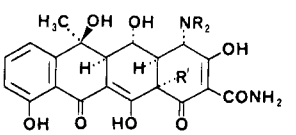
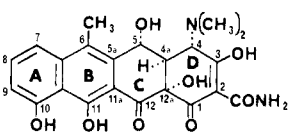
Abstract: The total synthesis of *dl*-terramycin is described in detail.

During recent decades, terramycin has proved to be a most useful antibiotic. As a member of the tetracycline family of antibiotics it possesses broad-spectrum, antibacterial effects and is active against rickettsias, certain large viruses, protozoa, and parasites.[2] Its structure was initially proposed in 1952 by a research group at Chas. Pfizer in conjunction with Woodward[3] several years after Finlay and colleagues announced preparation of the antibiotic by cultivation of *Streptomyces rimosus*.[4] The configuration proposed in 1953[3] was later revised after consideration of X-ray data[5] of terramycin and NMR spectra of a terramycin transformation product.[6] The structure of terramycin is thus depicted as **1**.



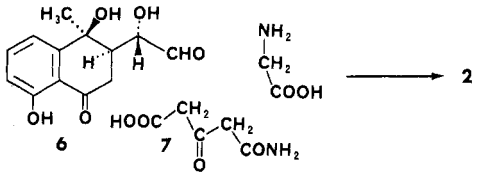
1, R=CH_3, R'=OH
2, R=H, R'=H



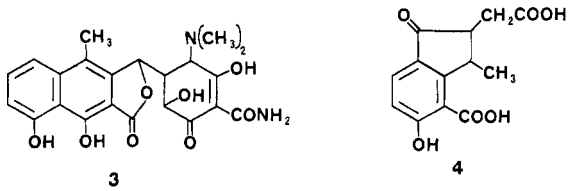
Anhydroterramycin

0002-7863/79/1501-0689$01.00/0

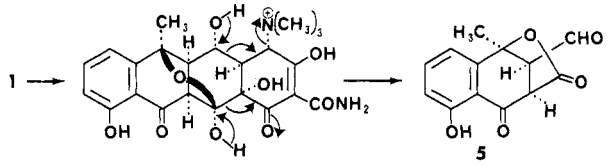
Scheme I



A major obstacle in the total synthesis of terramycin is the stereospecific introduction of many functional groups into the basic carbon nucleus.[7] Of even greater concern is the extreme chemical sensitivity of this molecule, particularly its lability in acidic and basic media. Dilute acid promotes dehydration to yield anhydroterramycin which suffers further cleavage and lactonization to apoterramycin (**3**). Mild alkali rapidly attacks the hydroxy groups at C-5 and C-6. The corresponding anions then cleave the polycarbonyl system to afford terracinoic acid (**4**) after a series of complex transformations.[3] Fragmentation



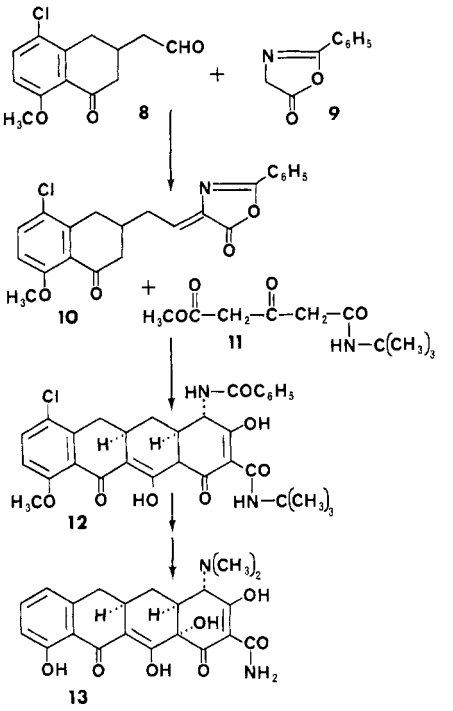
occurs even on exposure of terramycin to methyl iodide, whereupon the lactonized aldehyde **5** is produced.[8,9] In addition to these gross structural changes, terramycin undergoes a more subtle transformation. Acetic acid or aqueous buffers epimerize the nitrogen bearing C-4 to yield an equilibrium mixture of 4-*epi*-terramycin and terramycin.[10] Thus, to circumvent all these hazards, any synthetic approach must tread a fairly narrow pH range (pH 2–8) and avoid harsh reaction conditions unless the system were suitably stabilized during more vigorous maneuvers.



Our strategy for synthesis of terramycin was based on controlled condensation of a tetralone aldehyde such as **6** with a suitably activated glycine component and a derivative of 3-oxoglutaramic acid (**7**) (Scheme I). These subunits would be assembled to afford the tetracyclic product **2** which is related to terramycin by oxidation at C-12a and methylation of the C-4 amino group.

Substantial progress toward a solution to the problem of assembling the tetracyclic ring system had been made in a separate project carried out in our laboratory. This was the synthesis of the simpler and much more stable 6-deoxy-6-demethyltetracycline (**13**),[11] a fully biologically active degradation product of a naturally occurring tetracycline.[2,12] The synthetic principle utilized in that synthesis was very similar to that envisaged for terramycin. Three elements were essential: a tetralone aldehyde **8**, similar to but much simpler than the envisaged terramycin building unit **6**; glycine disguised as an oxazolinone **9**; and methyl *N*-*tert*-butyl-3-oxoglutaramate (**11**). These three building units were condensed stepwise via the oxazolone **10** to produce the tetracyclic compound **12**.[13] Several further transformations consisting of hydrolysis, oxidation, alkylation, and reduction culminated in the synthesis of **13** (Scheme II). It is quite obvious that glycine itself could not be used in a condensation reaction with tetralone aldehydes, as carbon–nitrogen bond formation would occur in preference to carbon–carbon bond formation. However, conversion of

Scheme II



glycine to a respective oxazolinone or thiazolinone activates the methylene group for rapid condensation with an aldehyde. The resulting unsaturated heterocycle **10** was then expected to be, and indeed was found to be, a very good Michael acceptor. In a remarkable series of transformations[11,13] **10** was observed to undergo reactions with derivatives of 3-oxoglutaric acid such as **11** in the presence of strong bases to produce the carbon skeleton fundamental to tetracyclines.

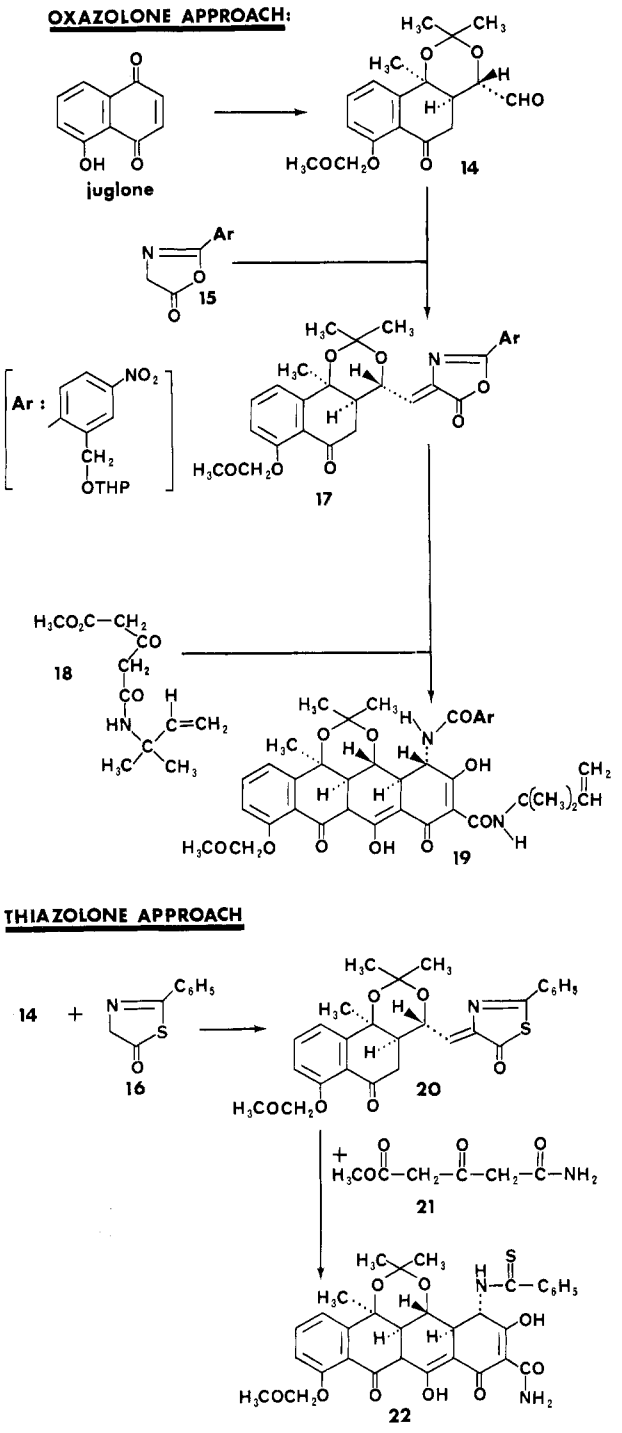
Any design of the terramycin precursor aldehyde had to take into account the strongly basic reaction conditions of the oxazolone condensation. Since both hydroxyl groups at C5 and C6 in terramycin interact intramolecularly with the polycarbonyl system under the influence of mild alkali to cause extensive ring fission, these functions were obviously not compatible with the strongly basic oxazolone annelation. Furthermore, it was suspected, and later verified experimentally, that ionization of the phenolic hydroxyl in ring D would retard reactions of the adjacent tetralone carbonyl group. Clearly, the hydroxyl groups in potential terramycin precursor **6** would require base-stable protecting groups which could be removed under mildly acidic conditions. The aldehyde was therefore protected with acetonide and methoxymethylene ether groups as in structure **14.**

The naturally occurring quinone, juglone, commonly encountered as the active staining principle of black walnut hulls, was chosen as our starting material for a stereochemically controlled synthesis of the key aldehyde **14.**

Two closely related approaches toward terramycin were then pursued almost simultaneously. The first approach utilized the oxazolinone **15** designed to accelerate acid hydrolysis of the C-4 amide function in tetracycline **19.** The second employed the simpler thiazolinone **16** and ultimately led to the complete elaboration of *dl*-terramycin. Scheme III summarizes these two pathways; the intermediates portrayed in the thiazolone route are the actual precursors of synthetic terramycin.

It is necessary at this point to emphasize that the terramycin problem is far more difficult than the prototype synthesis of **13.** Conversion of **12** into **13** required hot mineral acid for C-4 *N*-benzoyl cleavage and removal of the *tert*-butyl group from the C-2 carboxamido function, conditions which were out of the question with a sensitive terramycin precursor. Clearly, more subtle means for introducing the correct C-4 and C-2

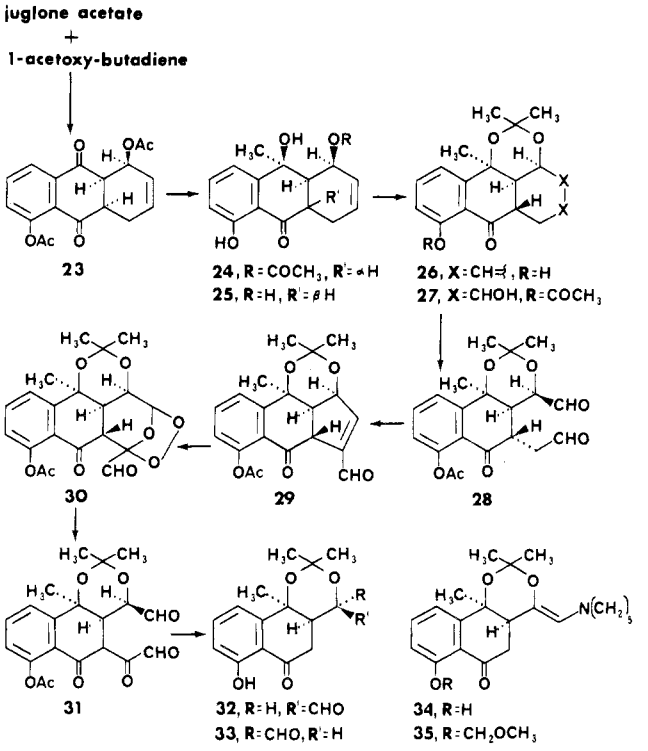
Scheme III



functionality would be necessary. These problems were expected to pose a lesser challenge than stereospecific construction of the key aldehyde **14,** however, and initial emphasis was placed on the synthesis of a properly functionalized and protected terramycin precursor aldehyde. At the time this work was begun the proposed structure of terramycin[1] differed in C-5 and C-4 stereochemistry from that later shown to be correct.[5,6] Therefore, the early part of our work was designed to prepare the unnatural C-5 epimer of aldehyde **14.** When the structure of terramycin was revised, it became necessary to change our efforts toward **14.** Fortunately, this proved to be a relatively easy matter since the natural C-5 epimer is the thermodynamically favored isomer.

Since the Diels–Alder reaction can be depended upon to provide products of predictable and well-defined stereochemistry, this reaction was utilized to introduce the requisite

Scheme IV



functionality onto juglone in a stereocontrolled fashion. Acetylation of juglone and subsequent reaction with 1-acetoxybutadiene led to the desired adduct **23** with remarkable selectivity[14,15] (Scheme IV). Excess of methylmagnesium iodide attacked **23** from the more accessible face to yield about 85% of **24** and a little 1-hydroxyanthraquinone. Saponification with sodium hydroxide and simultaneous inversion of C-11a[14] gave **25.** Anhydrous copper sulfate and dry acetone proved to be the medium of choice to produce acetonide **26** in quantitative yield. **26** was then acetylated and degraded by removal of the unwanted two-carbon unit from the right-hand ring. Oxidative cleavage of the double bond with catalytic amounts of osmium tetroxide and an excess of potassium chlorate[16] followed by treatment of the resulting crystalline mixture of cis glycols **27** with lead tetraacetate gave crystalline dialdehyde **28.** Ring contraction was finished by intramolecular Mannich reaction and elimination. A mixture of diazabicyclooctane, catalytic amounts of piperidine, and acetic acid in refluxing xylene or toluene gave **29** without isomerization of the newly formed double bond into conjugation with the tetralone carbonyl, a consequence which would have thwarted our plans. Ozonolysis of **29** produced crystalline **30** of partly unknown configuration. This ozonide cleaved with water to a substance which was equivalent for synthetic purposes to dialdehyde **31.** No aldehyde protons were visible in the NMR spectrum and a reproducible analysis could not be obtained. Nevertheless, the β-dicarbonyl system must be present since the substance was easily cleaved to the key terramycin precursor aldehyde. The vigor of alkali cleavage determined whether aldehyde **32** or epimerized aldehyde **33,** or a mixture of both, would be produced. Sodium carbonate at 0 °C provided largely **32;** more vigorous treatment at 40 °C converted **32** into epimer **33.** Large-scale synthesis of a mixture of **32** and **33** could then be carried out using this reaction sequence and final adjustments of C-5 stereochemistry could be made subsequently. Each step proceeded at an average of 80–90% for a respectable overall yield of 14% based on juglone.

Several considerations determined the next step in our almost completed synthesis of terramycin aldehyde **14.** First of all, separation of aldehydes **32** and **33** by chromatography on

preparative scale was difficult and tedious. The structural controversy concerning the configuration of the secondary hydroxyl group in terramycin had been resolved. Therefore, the stereochemistry present in the thermodynamically more stable aldehyde **33** would lead to the natural product. Secondly, experimental data from preliminary oxazolone condensations employing either aldehyde indicated that cyclization to tetracyclic systems required a more easily enolized tetralone carbonyl group in the parent aldehyde, confirming earlier suspicions that such might be the case. Hence, the phenol should be blocked by a base-resistant, acid-labile masking group, such as a methoxymethylene ether to facilitate cyclization. Base-catalyzed phenol alkylations of **32** or **33** turned out to be fruitless. However, the desired O-protection could be accomplished simultaneously with a final adjustment of C-5 stereochemistry by first preparing the base-resistant enamine **34** from the crude mixture of **32** and **33.** Reaction with piperidine in refluxing benzene gave the crystalline enamine in nearly quantitative yield and subsequent alkylation with chloromethyl methyl ether and sodium hydride yielded **35.** Furthermore, selective hydrolysis of the enamine function in **35** took place on moist silica gel to give exclusively the desired aldehyde **14.** No evidence for the presence of the epimeric aldehyde in the crude hydrolysis product was obtained, indicating that a stereospecific hydrolysis process was operative. Warm aqueous acetic acid cleaved **35** back to **33** in good yield, so aldehyde **14** was well suited for elaboration into a tetracyclic precursor of terramycin.

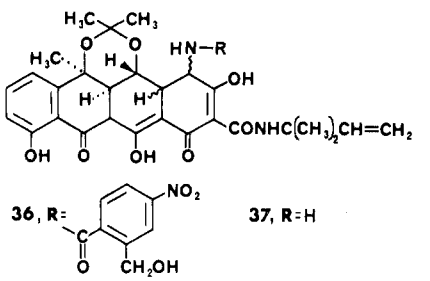
Up to this point, little evidence has been presented in support of the stereochemistry shown in the preceding structures, although ample data was accumulated in support of these structures. As discussed in an earlier publication,[17] the stereochemistry of adduct **23** is a consequence of the Diels–Alder selection rules. That the methyl group at C-6 in **24** was introduced in the desired stereochemical sense was deduced from numerous analogies in the literature.[18] The stereochemistry at the only remaining center, C-5, was easily established by NMR. Aldehyde precursors of the natural product (**14** and **33**) invariably possessed a large 11.0–11.5-Hz coupling constant for the hydrogens at C-5 and C-5a which is characteristic of a trans, diaxial arrangement of these hydrogens with respect to the acetonide ring. Direct descendants of the original juglone–acetoxybutadiene adduct **23** have the opposite stereochemistry at C-5, and exhibit a much smaller coupling of 3.0–3.5 Hz between C-5 and C-5a. Thus, the initially formed (thermodynamically less stable) aldehyde **32** has the C-5 and C-5a hydrogens cis with respect to the acetonide ring.

Further information was obtained when "dialdehyde" **31** was cleaved with potassium carbonate in D_2O: in **33,** three hydrogens were replaced by deuterium, whereas in **32** only the methylene protons next to ketone carbonyl were replaced. Clearly, the C-5 hydrogen had been exchanged by deuterium in **33** but not in **32.** This demonstrates that the mixture of deuterated **32** and **33** obtained in this experiment does not represent an equilibrium mixture and that **32** has retained the configuration of its precursors.

Having accomplished a viable synthesis of the terramycin aldehyde **14,** we had reached the first plateau in our work. Neither of the remaining two components required for the tetracyclic system, i.e., a suitably masked glycine fragment and a derivative of 3-oxoglutaramic acid, appeared to pose a major challenge. Nevertheless, we acquired much respect for the difficulties encountered in the synthesis and controlled introduction of these supposedly simple components.

As mentioned earlier, conversion of tetralone aldehydes into tetracycline derivatives employs Michael acceptors such as **17** or **20** (Scheme III). This strategy depends on eventual cleavage of the C-4 *N*-aroyl or *N*-thioaroyl group in tetracyclic products **19** or **22** under mild conditions which will not destroy the sensitive terramycin ring system. Another major consideration concerns the choice of a suitable 3-oxoglutaramate as the source of C-12, C-12a, C-1, and C-2. In the prototype synthesis of 6-deoxy-6-demethyltetracycline, *N-tert*-butyl-3-oxoglutaramate (**11**) was used for the one-pot condensation sequence (Michael addition to oxazolone; cyclization to form ring A; ring B closure). Ideally, the parent amide lacking an *N-tert*-butyl group (**21**) would have been used, but this substance could not be synthesized by methods which were successful for **11.** However, we found that an *N*-isopentenyl analogue **18** (Scheme III) could be prepared routinely. In model systems, the *N*-isopentenyl group could be cleaved with hot formic acid, conditions which are near the survival borderline of **12a**–deoxyterramycin derivatives. A synthesis of the parent amide **21** continued to elude our efforts, so attention was focused on using the *N*-isopentenyl 3-oxoglutaramate as the Michael addend.

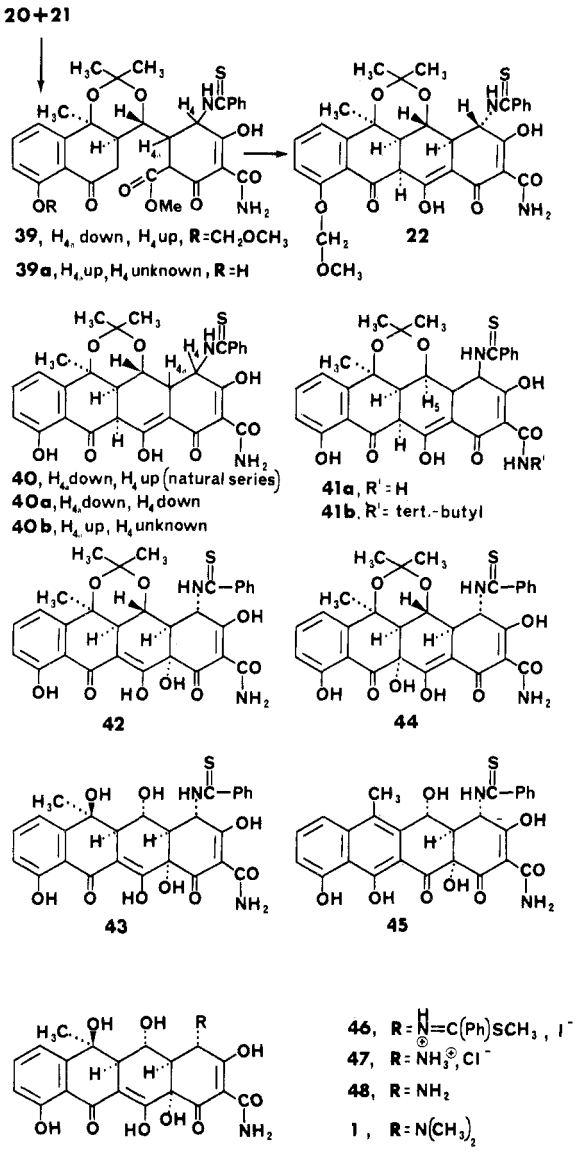
Among many potential solutions to the problem of mild cleavage of a C-4 *N*-aroyl group, we selected an approach which makes use of the known ability of neighboring hydroxyl to enhance the rate of amide cleavage.[19] A suitably functionalized oxazolinone **15** was therefore prepared (see Experimental Section) and condensation with terramycin aldehyde **14** gave the Michael acceptor **17** without difficulty. Subsequent condensation with *N*-isopentenyl 3-oxoglutaramate **18** in the presence of sodium hydride resulted in a tetracyclic molecule **19** which was characterized as the crystalline product **36** after hydrolytic cleavage of OTHP and OCH_2OCH_3 groups.



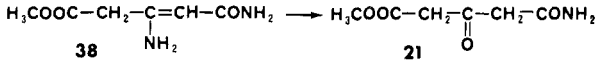
Upon heating in acetic acid, **36** was converted into a noncrystalline, tetracyclic derivative lacking the *N*-aroyl group which is presumed to be **37.** The aroyl group could be accounted for by isolation of 3-nitrophthalide (82%), the product expected from intramolecular amide cleavage by neighboring benzylic hydroxyl. However, more vigorous conditions which would be necessary to cleave the *N*-isopentenyl group (hot formic acid) resulted in mixtures which could not be separated. The isopentenyl cleavage could not be demonstrated, so the synthetic route based on **36** was abandoned. Nevertheless, we were encouraged because the visible–UV spectrum of **36** was closely analogous to the characteristic spectrum of 12a-deoxyterramycin.

Concurrently with efforts to utilize a neighboring group effect for assisted hydrolysis of *N*-aroyl terramycin precursors, exploration was underway in the sulfur series (Scheme III). Thus, it had been established in several systems[13] that thiazolinones behaved similarly to their oxazolinone analogues in condensations with aldehydes. Terramycin aldehyde **14** was unexceptional and condensed with 2-phenylthiazolin-5-one (**16**) in the presence of basic lead acetate to give the unsaturated thiazolone **20** in high yield (Scheme III). The NMR spectrum of **20** indicated clearly that the configuration at C-5 (J(H-5,5a) = 11.0 Hz) was retained during the condensation process. If this compound could be further transformed into a tetracycline by cyclization with an appropriate 3-oxoglutaramate derivative, our aim was then to cleave the thiobenzamide by S-methylation and mild hydrolysis of the resulting thioimidate.

Scheme V



On the same time scale, extensive efforts to prepare the parent 3-oxoglutaramate **21** were finally successful. Initially, **21** was isolated via $HgCl_2$-induced hydrolysis of the ethylene hemithioketal. Later, a more efficient synthesis was developed via enamine **38,** obtained by treatment of dimethyl 3-oxoglutarate in methanol with ammonia. Hydrolysis of a suspension of **38** in chloroform with concentrated hydrochloric acid left most of the water-soluble **21** in the organic phase and allowed easy isolation.



Unsaturated thiazolone **20** reacted readily in tetrahydrofuran solution with the lithium salt of **21** to form a mixture of dihydroresorcinol derivatives **39** having an intact A ring. Further addition of strong base (lithium or potassium *tert*-butoxide) and heating the mixture to reflux promoted ring B closure and the formation of tetracyclic substance **22** (Scheme V) which could be easily obtained in a crystalline state in modest yield (27%). This compound exhibited relatively weak absorption in the visible region (λ_{max} 452 nm, ϵ 9900, in 0.1 M methanolic sodium tetraborate), but the more familiar spectral curves typical for 12a-deoxyterramycin were produced after hydrolysis of the methoxymethylene ether in warm, aqueous acetic acid. The hydrolysis product **40** displayed intense absorption at 440 nm (ϵ 34 300) in sodium tetraborate solution which was taken to mean that the molecule can form a planar, fully conjugated borate complex along the β-dicarbonyl periphery. As in **39,** extended conjugation in **40** was not present in 0.01 M methanolic HCl, and C-11a was protonated as shown in the tautomer represented by structure **40** (Scheme V).

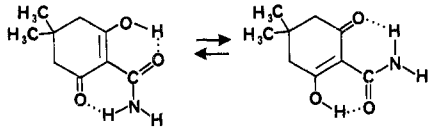
Low solubility of the key tetracyclic intermediate **40** in most solvents made NMR spectra difficult to obtain. Deuterated dimethyl sulfoxide was satisfactory, however, and the 60-MHz spectrum of **40** in this solvent was extremely informative even though solvent partially obscures a doublet of doublets at 2.5 ppm. The latter was resolved using nitrobenzene as solvent. Since the cyclization reaction leading to **22** could yield as many as four stereoisomers it was essential to show that the isolated tetracyclic product had the C-4, C-4a stereochemistry of terramycin. Accordingly, a complete assignment of methine proton signals was made with the help of decoupling studies. In the dimethyl sulfoxide spectrum, a low-field doublet (10.29 ppm, J = 9 Hz) was obviously due to the thioamide proton, and the carboxamide function logically accounted for the adjacent broadened two-proton signal at 9.26 and 9.40 ppm. Since three other very low field signals, undoubtedly arising from hydrogen-bonded protons, were present, it follows from the UV and NMR spectra that C-12a is trigonal, thus accounting for the third (enolic) hydrogen-bonded proton. Easily exchangeable protons were replaced with deutrons by addition of deuterium oxide and a trace of pyridine. The new spectrum no longer contained any low-field absorption below the aromatic region nor a doublet at 4.25 ppm (J = 5 Hz) which was present prior to deuteration. Also, single proton absorptions at 5.99 and 2.50 ppm had collapsed from four-line signals each to doublets with J = 1.25 and 11.0 Hz, respectively. It was then a simple matter to assign resonances, extract coupling constants, and ascertain the molecular geometry of **40.**

The 5.99-ppm absorption in dimethyl sulfoxide solution was readily explicable on the basis of chemical shift and chemical decoupling by deuteration of the adjacent thioamide group as due to H-4 coupled to H-4a by 12.5 Hz. Obviously, the doublet at 4.25 ppm belonged to H-11a. Coupling constants at 8.0 and 12.5 Hz were apparent in the splitting pattern of a single proton resonance at 3.17 ppm which was therefore due to H-4a, and the H-5 methine signal was located nearby at 4.09 ppm (J = 8 and 11 Hz). This left the H-5a resonance to be found partially obscured by solvent absorption at 2.50 ppm. The coupling values for this proton were elucidated more conclusively in other solvents, i.e., deuteriochloroform, deuterionitrobenzene, and hexafluoroacetone·1.6D_2O. Decoupling experiments performed at 100 MHz verified the relative assignments and coupling constants.

The significance of these data was soon appreciated in that the conformation and relative stereochemistry of **40** could be defined precisely. Ring A and B lay nearly in one plane; rings B and C were cis fused to provide a practically strain-free acetonide linkage (Scheme V). No other conformation or relative stereochemistry can account for the large coupling constants along the C-4,4a,5,5a periphery, so the single most important conclusion follows that **40** must have the same stereochemistry as terramycin itself. This conclusion was verified by eventual conversion of **40** into the natural product.

The NMR spectra of **40** and related structures are amazingly clear-cut when proper solvents and temperature conditions are chosen. However, these molecules can, and generally do, exist in solution as isomers in epimeric and tautomeric equilibrium. In less polar solvents such as chloroform-*d* and nitrobenzene-d_5, the NMR spectra at room temperature are exceedingly complex, and suggest the presence of an equilibrium mixture of two isomers possessing a relatively high energy barrier to interconversion. The most satisfactory explanation

Table I. Coalescence Temperature as a Function of Solvent for Carboxamidodimedone



solvent	T_c, °C
pyridine-d_5	<−30
dimethyl sulfoxide-d_6	16
chloroform-d	≫40
nitrobenzene-d_5	145
o-dichlorobenzene	>170

is hindered roatation about the bond joining C-2 to the carboxamide function giving rise to a mixture (approximately 3:2) of isomers. This bond is known to be unusually short from X-ray data[5,6] and the existence of two relatively stable rotamers has long been suspected.[3,5d,20]

Coalescence temperatures (T_c) for the methylene singlets in the simple model 2-carboxamido-5,5-dimethylcyclohexane-1,3-dione[21] have been determined (Table I). These measurements reinforce our interpretation of the solvent- and temperature-dependent spectra of **40** and imply that the rotational barrier is relatively high in solvents of low polarity and not detectable at room temperature in highly polar solvents.[22]

Several other products were formed in the condensation between **20** and **21.** To facilitate characterization, the mother liquors from crystallization of **22** were treated with hot acetic acid to remove the methoxymethyl ether group. Chromatography of the resulting mixture gave two new compounds in addition to a small amount of **40.** One of the isolated side products was clearly tetracyclic, and differed only slightly from **40** in UV properties. This substance is tentatively assigned the 4-epi structure **40a,** based on the reasonable assumption that stereochemical changes involving other asymmetric centers would distort the tetracyclic framework and thereby would affect the UV chromophore more strongly.

The second side product analyzed correctly for a tricyclic structure such as **39a** (R = H). Upon treatment with $KOC(CH_3)_3$ under forcing conditions, **39a** cyclized to give a third tetracyclic isomer **40b** which is distinguished by a modified UV chromophore relative to **40** or **40a.** The differences in UV absorption are most striking in basic methanol. Both **40** and **40a** are yellow and absorb strongly at 430 nm (ε ca. 15 000), while **40b** has the corresponding λ_{max} at 368 nm and is nearly colorless in CH_3OH–0.01 M NaOH. Upon addition of borate, **40b** does absorb at 430 nm, but not as strongly as do **40** and **40a.** According to molecular models, the unnatural C-4a epimer **40b** has greater difficulty attaining the fully conjugated chromophore responsible for absorption in the 430-nm region.

A reasonable alternative structure **41a** could be considered for **40b** if the starting thiazolone **20** is subject to C-5 epimerization prior to condensation with **21.** However, we had earlier prepared a tetracyclic substance **41b** (C-4,4a stereochemistry uncertain) in the C-5 epi series by converting the unnatural C-5 aldehyde isomer **32** into a thiazolone which was then condensed with *N-tert*-butyl-3-oxoglutaramate (**11**). The resulting crystalline C-5 epi tetracycle **41b** has an intense chromophore at 465 nm in methanolic borate. Since the presence or absence of the *N-tert*-butyl substituent has little effect on UV spectra of closely related compounds in the 6-deoxy-6-demethyl series, we can safely conclude that **40b** does not have the same C-5 stereochemistry as **41b.** Clearly, the thiazolone condensation and cyclization to tetracyclic products occur without epimerization at C-5.

Based on the above findings, it is also quite clear that there is little control of C-4,4a stereochemistry in the condensation between **20** and **21.** All possible C-4 and C-4a epimers of tricyclic **39** are probably formed to some extent. Fortunately, the desired tetracyclic isomer **22** appears to be formed more rapidly than are its C-4 or C-4a epimers under basic conditions, and product isolation is relatively simple. Whether the C-4 stereochemistry in **22** reflects a kinetic or thermodynamic preference has not been established.

Returning to synthetic considerations, conversion of our highly functionalized **40** into terramycin could be envisaged to proceed by several routes. In theory, three modifications of the molecule would be necessary, i.e., hydrolysis of the acetonide function, conversion of the thiobenzamido group into a dimethylamino group, and selective introduction of a hydroxy group at C-12a cis relative to the hydrogen at C-4a. For practical reasons, it would be desirable to retain the neutral nitrogen function at C-4 as long as possible rather than to deal with amines possessing polar zwitterionic properties. The acetonide group imparts considerable stabilization to the molecule relative to the free hydroxyl functions and should be retained for this reason as long as convenience would permit. Aside from its base-stable property, the ketal also stabilizes the molecule with respect to thermal degradation. During variable-temperature NMR studies in dimethyl sulfoxide-d_6 solution, the ketalized molecule **40** appeared to be stable for several hours at 125 °C. In contrast, terramycin was rapidly dehydrated at temperatures above 50 °C. A fair proportion of the enhanced stability of the acetonide must be attributed to the equatorial orientation of the C-6 oxygen function. The carbon–oxygen bond is less easily broken in this orientation, in contrast to terramycin, where an axial carbon–oxygen bond is easily attained. In the latter molecule, the aromatic ring is properly oriented to stabilize the developing orbital at C-6 during dehydration.

A more disturbing reflection of acetonide stability was encountered at this point when it became clear that the acetonide function in **40** was also resistant to acid hydrolysis. A variety of aqueous acidic conditions were found to have no effect on the ketal without simultaneously aromatizing ring B. Providentially, hydroxylation of **40** by gaseous oxygen proceeded rapidly and efficiently in a medium consisting of sodium hydride, triethyl phosphite, tetrahydrofuran, and dimethylformamide. In addition, a small amount of water seemed to be essential to initiate this reaction. But the biggest dividend of this oxidation was realized when the hydroxylated substance **42** was exposed to dilute hydrochloric acid in methanol. The acetonide linkage in **42** was apparently much more labile than in the unhydroxylated precursor **40,** for hydrolysis proceeded rapidly to give the deketalized species **43.** In fact, the ketal function in **42** was so sensitive to moisture that isolation was difficult. Instead, it was expedient to immediately subject the major hydroxylation product **42** (52%) and minor product **44** (14%) to weak acid and separate the deketalized, highly polar substance **43** from the less polar compound **44** retaining the acetonide function under these conditions. Separation was easy by chromatography on polyamide and crystallization.

The electronic spectra of **43** in 0.01 M NaOH–CH_3OH and in 0.01 M HCl–CH_3OH were very similar to the corresponding spectra of terramycin if allowance is made for the thioamide absorption in **43.** The virtual identity of the spectra after subtraction of the thiobenzamide absorption with the spectra of terramycin was strong evidence for hydroxylation occurring in the desired manner, i.e., stereospecific introduction of a hydroxyl at C-12a resulting in a cis fusion of rings A and B.

Mild acid treatment converted **43** into anhydro derivative **45** possessing visible absorption at 425 nm. This was additional evidence for our structural assignment since anhydroterramycin and dedimethylamino-12a-*epi*-anhydroterramycin have

Table II. NMR Spectra of Synthetic Tetracyclines

compd	**22**		**40**			**40b**			**40a**	**41b**	**44**		**43**	synthetic (±)-terramycin		authentic terramycin	
solvent	*e*	*e*	*b,f*	*g*	*e*	*b,h*	*g*	*d,e*	*b,d,f*	*b,d,h*	*d,i*	*e*	*j*	*j,l*	*e*	*e*	*k*
temp[a]				80 °C			56 °C										
acetonide	0.65	0.60	0.78	0.82	0.85	0.90	1.00	0.68	0.78	1.05	0.63						
CH_3	1.12	1.12	1.28	1.30	1.33	1.43	1.60	1.35	1.44	1.48	1.13						
C_6–CH_3	1.63	1.63	1.73	1.72	1.69	1.58	1.80	1.69	1.72	1.80	1.79	1.73	2.32	2.15	1.73	2.00	
H_{5a}	2.44	2.50	2.50	2.48	2.62	2.48	3.03	2.53	2.49	2.60	2.40	2.82	3.35	3.38	2.83	3.00	
H_{4a}	3.16	3.17	3.18	3.00	3.50	3.47	3.55	3.08	3.13	~4.0	3.25	2.63	3.47	3.07	2.44	2.77	
H_5	4.08	4.09	4.50	4.45	4.25	4.28	4.48	3.80	3.96	4.57	4.11	3.9	4.98	5.32	4.43	4.9	
H_{11a}	4.08	4.32	3.94	3.95	4.29	3.85	4.08	4.30	3.84	15.52		15.0	*c*	*c*	*c*	*c*	
H_4	6.00	5.99	6.27	6.18	5.93	~6.2	6.45	5.85	6.13	5.28	6.01	6.18	~7.5	4.05	3.42	3.77	
			6.05						5.96	5.56							
Ar-H	6.8–8.0	6.8–8.0	6.7–8.3	6.7–8.2	6.9–8.0	6.8–7.9	6.8–8.2	6.8–7.8	6.8–7.6	6.8–8.1	6.7–8.0	6.8–8.0	7.0–8.3	6.8–7.7	6.7–7.7	6.8–7.7	
$CONH_2$	9.20	9.26	7.0	8.24	9.11	6.4	8.22	9.05	6.3		8.95	9.0–9.2	9.8–10.0	*c*	8.74–9.07	8.5–9.40	
	9.40	9.40	9.50	9.50	9.32	~9	9.47	9.28	9.31	9.91	9.40			*c*			
			9.86						9.92	10.42							
CSNH	10.27	10.29	8.60	9.15	10.47	~7	9.78	10.3	7.3	8.75	9.88	9.83	11.08				
										8.57							
C_{10}–OH		11.82	12.12	12.00	12.05	11.85	12.17	12.16	11.82	12.30	11.6	11.60	*c*	*c*	*c*	*c*	
			12.10							12.34							
H_{12a}	14.7	14.73	15.18	14.5	14.2	~15	14.5	14.6	14.8	3.86	15.4						
			13.33						13.2	3.67							
C_3enol	17.3	17.30	17.7	17.4	?	~15	17.9	17.7	?	18.10	17.2	17.9	*c*	*c*	*c*	*c*	
			16.9						17.1	17.26							
misc	5.22, OCH_2									1.45	6.67			2.65	2.44	2.64	
	3.43, OCH_3									1.42	C_{11a}–OH			$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	
										$C(CH_3)_3$							
							Coupling										
temp[a]			120 °C				56 °C					84 °C			60 °C		
$J_{5a,11a}$		5	5		5	4.5	5	5	5								
$J_{5a,5}$		11	11.5		12.5	12.5	12.5	11.5	11.5	2.5	12	8	7.5	3.0	4	3	
$J_{5,4a}$		8	8		8	8.5	8.5	9	9	1.5	8	8.5	10.5	1.5	3	1.5	
$J_{4a,4}$		12.5	13		4	3.5	4	4	4	6	12.5	4.5	4.5	10.5	9.5	10	
$J_{4,NH}$		9	9		9	9	9.5	9.5	9.5	4.5	9	9	9				
$J_{4a,12a}$										6							

[a] 35 °C except where noted. [b] Separate signals for each rotamer observed in these solvents. [c] Not assigned. [d] 100-MHz spectrum. [e] CD_3SOCD_3. [f] $C_6D_5NO_2$. [g] CD_3SOCD_3–$C_6D_5NO_2$ (7:100). [h] $CDCl_3$. [i] $CDCl_3$–$CD_3SO_2CD_3$ (1:1). [j] C_5D_5N. [k] $CD_3SO_2CD_3$–$C_6D_5NO_2$ (14:100). [l] Synthetic and authentic terramycin have identical spectra in C_5D_5N both recrystallized from acetone. [m] Spectra of **40** under different conditions are reproduced below in the microfilm edition.

absorption maxima at 425 and 413 nm, respectively.[3,9]

It was clear from both the electronic and NMR spectra of **43** that a number of drastic conformational changes had occurred in hydroxylation and deketalization of our tetracyclic skeleton. No longer constrained by the isopropylidene bridge, the molecule was now much more flexible. With conjugation across rings A and B rendered impossible by the newly introduced hydroxy group, enolization at C-11a could occur, extending conjugation of the tetralone system to include ring B. This was evident from the chemical shift value of 15 ppm for the 11a hydrogen in the NMR spectrum (Table II) and also quite obvious in the electronic spectra in acidic methanol [λ_{max} 360 nm (ϵ 15 400), and 315 (ϵ 24 700) for **43** and **40**]. The presence of a fully conjugated B, C, D ring chromophore in both terramycin and **43** suggested that these three rings had a nearly coplanar chromophore. However, the NMR data intimated that the thiobenzamide compound **43** possessed a very different conformation from both its ketal precursor **40** and from terramycin itself, at least in polar solvents used for gathering spectral data.

The small 4.5-Hz coupling constant relating hydrogens C-4 and C-4a in the NMR spectrum of **43** was particularly perplexing. In both precursor **40** and ultimate product, terramycin, this coupling is large (12.5 Hz, Me_2SO-d_6, in compound **40**; 9.5 Hz, Me_2SO-d_6 or pyridine-d_5 in terramycin) implying a corresponding dihedral angle on the order of 180° in both molecules. Since experience in our laboratory had led us to believe that the small coupling constant of 4–5 Hz was nearly always associated with the series of molecules epimeric at C-4, we had grave reservations concerning retention of configuration at this center during the sequential oxidation and hydrolysis. However, molecular models indicated that a new conformation is possible for **43** in which the planar hydrogen-bridged diketocarboxamide moiety of ring A is skewed in an almost perpendicular fashion to the relatively coplanar B, C, D rings. This geometry would satisfy the dihedral angle requirements suggested by the C-4, C-4a coupling.

In addition to **42**, another hydroxylation product **44** was formed in considerably lower yield which proved to be relatively resistant to acetonide hydrolysis. Consideration of analytical, spectral, and chromatographic data led to structural assignment **44** which amounts to formal insertion of oxygen into the C-11a carbon–hydrogen bond of **40**.

We were now in position to complete the synthesis of terramycin. Modification of the C-4 nitrogen function was anticipated to lead directly to terramycin or 4-*epi*-terramycin if epimerization had indeed occurred during hydroxylation.

Methylation of the thioamide **43** at sulfur was readily accomplished with methyl iodide in tetrahydrofuran at room temperature. The hydriodide salt **46** was not purified but subjected to hydrolysis in aqueous hydrochloric acid to the amine hydrochloride **47**. Ultraviolet spectra of **47** were qualitatively identical with those of terramycin. A more exact comparison was not made because **47** was obtained only in a crude state. The two-step dethioacylation proceeded in yields approaching 80%, although the procedure for isolation of **47** generally produced material contaminated by small amounts of the corresponding anhydro compound. Finally, alkylation of the crude amine hydrochloride **47** with dimethyl sulfate in the presence of *N,N*-diisopropylethylamine (Hünig's base) gave racemic terramycin in 33% yield (23% from crystalline **43**) after elution from polyamide with chloroform and crystallization from acetone. In light of more recent work in our laboratory, the method of choice for dimethylation of amine **47** would have to be a reductive alkylation procedure employing sodium cyanoborohydride and formaldehyde.[25] The isolation of terramycin rather than 4-*epi*-terramycin was excellent evidence that **43** had the unepimerized structure.

Synthetic terramycin was isolated as light yellow crystals containing 0.8 mol of acetone (NMR) after thorough drying. Terramycin obtained by fermentation also contained about 1 mol of acetone when recrystallized from this solvent. Synthetic and authentic terramycin were then compared. The NMR spectra in pyridine-d_5 were identical if taken at the same concentration. Ultraviolet spectra were superimposable. The identity was further established by mass spectral data and chromatographic behavior on polyamide using several different solvent systems. These chromatographic systems differentiated clearly between terramycin, 4-*epi*-terramycin, *N*-demethylterramycin, and *N,N*-didemethylterramycin **48**. A bacteriological assay[26] showed synthetic *dl*-terramycin to be about 50% as active as terramycin from *Streptomyces rimosus*.

Experimental Section

Proton magnetic resonance spectra were taken with a Varian A-60 NMR spectrometer, if not mentioned otherwise. Chemical shifts (δ in ppm) are reported relative to the internal standard tetramethylsilane. Melting points were detected on a Kofler hot-stage microscope and are uncorrected.

Juglone Acetate. (The preparation was an adaptation of the method of Fieser.)[27] Juglone (50 g, 0.287 mol) was dissolved with heating in 500 mL of acetic anhydride. This solution in a 1-L Erlenmeyer flask was cooled to −10 °C. Some juglone precipitated, but redissolved on addition of 5 mL of concentrated sulfuric acid to the cold solution. The temperature rose to about 15 °C as acetylation proceeded and juglone acetate precipitated. Yellow leaflets of the acetate continued to precipitate on further stirring at 0 °C. The product was filtered and washed with acetic acid and then water. The combined mother liquor and acetic acid wash were stirred with 2 L of ice water to destroy acetic anhydride and precipitate a second crop of brown crystals. This crop was recrystallized once from ethyl acetate and combined with the first crop of juglone acetate which was pure enough for the Diels–Alder reaction, yield 51.5 g (83%), mp 153–154 °C (lit.[27] 153–154 °C).

Diene Adduct 23. Juglone acetate (100 g, 0.463 mol) and 1.2 L of benzene were placed in a 2-L, round-bottomed flask fitted with stillhead and condenser. Moisture was removed by azeotropic distillation, a few milligrams of methylene blue and 200 mL of acetoxybutadiene were added, and the mixture was refluxed for 3 h. The hot solution was filtered and then concentrated in vacuo to ¼ of its original volume. The crystals which formed were filtered off and the mother liquor diluted with about 100 mL of ether to precipitate more adduct as light gray crystals. After filtration of this crop, solvent and acetoxybutadiene were completely stripped off to yield a brown oil. The isomeric diene adduct (mp 84–86 °C) was largely obtained on cooling an ether solution of this oil in a freezer. While the first crop of the desired adduct was pure enough for subsequent steps, the second crop required recrystallization from benzene, yield 91.1 g (60%) of **23**:[29] mp 174–176 °C; IR ($CHCl_3$) 1700, 1734 cm^{-1}.

Grignard Product 24. Diene adduct **23** (20 g, 0.061 mol) was dissolved in 1.8 L of warm toluene in a 2-L, three-necked flask and cooled to −70 °C. An ethereal solution (50 mL) of methylmagnesium iodide (from 8.89 g of magnesium, 6 equiv) was added over 30 min to the cold, stirred solution. The temperature was maintained below −65 °C during the addition and for 3 h afterwards. The mixture was then allowed to warm to −10 °C and was poured into a 5-L Erlenmeyer flask containing 35 mL of concentrated hydrochloric acid and 2 L of ice water. The organic layer was separated and the aqueous phase was washed twice with 200 mL of ether. The combined organic extracts were washed with water to neutrality, dried, and concentrated in vacuo to a mass of grayish crystals. The crystals were washed with ether and recrystallized from benzene. The yield was 15.1 g (82%) of **24** (mp 162–165 °C) giving no depression with authentic product prepared previously.[29]

Saponification of 24 (Preparation of 25). 24 (20 g, 0.067 mol) was dissolved in 300 mL of aqueous potassium hydroxide (18.6 g) solution and kept at room temperature for 18 h. On cautious acidification with dilute HCl, the oil which initially separated gradually crystallized in the course of about 1 h. The product was taken up in 500 mL of warm chloroform and the water removed in a separatory funnel. The organic layer was washed with water, dried, and concentrated to about 100 mL (aspirator). On dilution with petroleum ether, the product **25** was precipitated (14.5 g, 84%), mp 155–157 °C, no melting point depression with authentic material.[29]

Acetonide 26. Triol **25** (15 g, 0.058 mol), anhydrous cupric sulfate (dried at 260 °C for 2 h in vacuo), and 1.5 L of dry acetone were stirred in a 2-L flask under nitrogen at room temperature for at least 48 h. The progress of the reaction was followed by observing the hydroxyl absorption in the IR (2.8 and 2.95 μ). When the bands had disappeared completely, the copper sulfate was filtered off and washed with acetone. Solvent was evaporated from the filtrate in vacuo to give a residue which was recrystallized from ether to yield 14.7 g (84%) of **26,** mp 145–146 °C. An analytical sample[28] was obtained after three more recrystallizations: mp 146–147 °C; IR ($CHCl_3$) 1625 cm^{-1}; UV (methanol) λ_{max} 217 nm (ϵ 18 300), 256 (8200), and 333 (4400).

Acetylation of 26. 26 (10 g, 0.033 mol) was dissolved in 50 mL of acetic anhydride and 5 g of sodium acetate was added. The mixture was heated on a steam bath for 2 h and poured onto ice. The acetate crystallized from the stirred mixture over 2 h and was filtered off. The crystals were washed with water until the wash was no longer acidic and then dried in a vacuum desiccator over potassium hydroxide to give 10.9 g (95%) of the acetate of **26:** mp 145–147 °C (recrystallized from ether);[28] UV (methanol) λ_{max} 242 nm (ϵ 8200) and 290 (1700).

Diol 27. A solution of 3.4 g (0.01 mol) of the acetate of **26** in 50 mL of dry tetrahydrofuran was combined with a solution of potassium chlorate (2 g, 0.015 mol) and osmium tetroxide (125 mg, 0.5 mmol) in 25 mL of water. After the mixture was stirred at 50 °C for 5 h, the tetrahydrofuran was removed in vacuo. Glycol **27** was then filtered off and washed with water. Recrystallization from ether gave 3.3 g (89%) of **27:** mp 183–191 °C;[28] UV λ_{max} 241 nm (ϵ 9100) and 289 (1860).

Dialdehyde 28. Diol **27** (105 g, 0.279 mol) was dissolved in 5 L of acetone (distilled twice from anhydrous potassium carbonate) at 40 °C in a 12-L, three-neck flask equipped with stirrer, condenser, and drying tube; 157 g (0.355 mol) of lead tetraacetate (dried for 8 h at 40 °C under vacuum) was added at once and stirring was maintained for 1 h at 40 °C. The heating bath was removed, and 5 L of hexane was added. After 10 min the lead salts were filtered off and washed thoroughly with 2 L of a 1:1 benzene–chloroform mixture. The organic phase was combined, evaporated in vacuo, and redissolved in 2 L of chloroform. The chloroform solution was washed twice with water, dried, and evaporated to yield the dialdehyde as a yellow oil. This material was pure enough for the next step. For analysis, the dialdehyde was crystallized three times from ether:[28] mp 135–138 °C; IR ($CHCl_3$) 2815, 2715, 1750, 1720, 1680, 1590 cm^{-1}; NMR ($CDCl_3$) δ 9.74 (s, 1 H), 9.65 (s, 1 H), 7.7–6.9 (m, 3 H), 4.69 (d, J = 4 Hz, 1 H), 3.6 (m, 1 H), 2.8–2.4 (m, 3 H), 2.28, 1.69, 1.53, and 1.32 (s, 3 H each).

Cyclization of 28 to Unsaturated Aldehyde 29. The crude oily dialdehyde **28** from above was dissolved in 2.1 L of xylene (reagent grade) and separated into seven aliquots of 300 mL. Each aliquot was placed into a 3-L, three-neck flask equipped with a water separator and a condenser. More xylene was added to a volume of 750 mL and the mixture was brought to reflux. A solution of diazabicyclooctane (4.5 g, 0.025 mol) in acetic acid (75 mL) was added and followed by a solution of piperidine (0.5 mL) in xylene (25 mL). The mixture was then refluxed for 7 min. Heating of the dark red solution was stopped, and the mixture cooled for a few minutes in an ice bath followed rapidly by washing five times with water. The organic phase was dried, and the solutions from seven such procedures were combined and evaporated at 60 °C on a flash evaporator. The last traces of xylene were removed in a rotatory evaporator under vacuum to yield a dark, partly crystalline gum. The product was heated with ether (250 mL) until all gummy material had dissolved and allowed to crystallize at room temperature. The brown, crystalline material was filtered off and washed with chilled ether containing 20% of benzene to give 51 g (52%) of unsaturated aldehyde **29,** mp 157–162 °C. An analytical sample was prepared by recrystallization (three times) from ether: mp 162–165 °C;[28] IR ($CHCl_3$) 2740, 1750, 1700 cm^{-1}; UV (methanol) λ_{max} 248 nm (ϵ 7600) and 290 (1800); NMR ($CDCl_3$) δ 9.74 (s, 1 H), 5.21 (dd, J = 8 and 2 Hz, 1 H), 4.12 (dd, J = 8 and 2 Hz, 1 H), 3.17 (t, J = 8 Hz, 1 H), 2.22, 1.56, 1.46, and 1.40 (s, 3 H each).

Ozonolysis of Unsaturated Aldehyde 29. A Wellsbach T-23 ozonator was equilibrated for 2 h at the following settings: "sample" flow rate, 0.06; "ozone" flow rate, 0.02; pressure, 8 psi; voltage, 62 V. The sample ozone flow rate was titrated as follows. Ozone was passed through 200 mL of concentrated aqueous potassium iodide for 3 min. The solution was acidified with sulfuric acid and rapidly titrated with 0.1 N sodium thiosulfate to the first disappearance of iodine color, 14.2 mL being required in this instance. Thus, 11.4 mg of ozone per min was available. A solution of the unsaturated aldehyde **29** (5 g) in chloroform (300 mL, distilled/phosphorus pentoxide) was chilled to −50 °C. The titrated ozone flow was passed through for 65 min (10% excess) and excess ozone removed by passing nitrogen through the cold solution. The mixture was allowed to come to room temperature and used directly in the next reaction. An analytical sample of the ozonide **30** was obtained by removal of the solvent in vacuo and crystallizing several times from ether:[28] mp 104 °C dec; IR ($CHCl_3$) 1750, 1730, 1675 cm^{-1}; UV λ_{max} (in ether) 245 nm (ϵ 6900) and 290 (1700).

Hydrolysis to "α-Keto Aldehyde 31". Water (25 mL) was added to the chloroform solution from above directly after ozonolysis of unsaturated aldehyde **29,** and the two-phase mixture was allowed to stand at room temperature for 2 days with occasional shaking. The aqueous phase was then separated, and the chloroform layer was dried over sodium sulfate and evaporated at 40 °C. The pale yellow residue was taken up in ether containing a little chloroform, and the solution allowed to evaporate slowly at room temperature. Several crops of cream-colored crystals were collected over 2 days, total yield, 3.7 g (68% over two steps from **29**), mp 130–138 °C. **31** was somewhat unstable at room temperature, but could be stored for months in a refrigerator without deteriorating. Satisfactory analysis could not be obtained. IR ($CHCl_3$): 1750, 1640 cm^{-1}. UV: λ_{max} (in 0.01 N methanol) (NaOH) 220 nm (ϵ 23 800), 250 (11 800), and 348–352 (11 500). NMR ($CDCl_3$): δ 5.8 (s, 1 H, exchanged by D_2O), 7.8–6.9 (m, 4 H, 1 H exchanged by D_2O), 5.62 (broad d, J = 2 Hz), 4.39 (t, J = 2 Hz), 2.8 (d, J = 2 Hz), 2.30, 1.93, 1.53, and 1.18 (s, 3 H each).

Aldehydes 32 and 33 (Mixture). The keto aldehyde **31** (5 g, 0.013 mol) was dissolved in methylene chloride (1 L). A solution of 0.5 N sodium carbonate (500 mL) was added and the mixture was stirred vigorously for 2 h. The red water layer was separated, washed with methylene chloride, and discarded. The combined organic phases were washed with water, dried over sodium sulfate, and evaporated. The yellow residual oil crystallized from ether (20 mL) to yield two crops (3.0 g, 85%) of a mixture of **32** and **33,** melting range ca. 120–160 °C. Spectra are discussed below.

Unepimerized Aldehyde 32. The keto aldehyde **31** (0.5 g, 1.3 mol) was dissolved in cold ethyl acetate (150 mL). A solution of 0.5 N aqueous sodium carbonate, chilled at 0 °C, was added, and the reaction mixture stirred vigorously at 0 °C for 4 h. The same workup as above yielded 0.11 g (30%) of **32,** mp 143–145 °C. The analytical sample was recrystallized twice from ether: mp 143–145 °C;[28] IR ($CHCl_3$) 1730, 1635 cm^{-1}; UV in methanol λ_{max} 258 nm (ϵ 9400), 335 (4500); NMR ($CDCl_3$) δ 12.28 (s, 1 H), 9.64 (s, 1 H), 7.7–6.8 (m, 3 H), 4.71 (d, J = 3.5 Hz), 3.3–2.3 (m, 3 H), 1.82, 1.64, and 1.47 (s, 3 H each).

In an earlier publication, the C-5 stereochemistry of this aldehyde was incorrectly assigned.[17] The C-11a dideuterated aldehyde **32** was prepared in the same way, mp 140–143 °C, using sodium carbonate in deuterium oxide. The infrared spectrum of the deuterated aldehyde showed differences in the fingerprint region from the nondeuterated aldehyde **32,** but otherwise was the same. The complex NMR signals between 3.3 and 2.3 were replaced by a broad doublet at δ 2.6 (J = 3.5 Hz, 1 H) as the only change.

Epimerized Aldehyde 33. The keto aldehyde **31** (0.5 g, 1.3 mol) was dissolved in ether (100 mL) and a solution of 2 N sodium carbonate (50 mL) was added. The mixture was refluxed for 45 min and worked up as described before. A yield of 80 mg (20%) of **33** was obtained, mp 167–171 °C. The analytical sample was recrystallized twice from ether: mp 171–173 °C;[28] IR ($CHCl_3$) 1730, 1635 cm^{-1}; UV in methanol λ_{max} 259 nm (ϵ 8300) and 335 (3900); NMR ($CDCl_3$) δ 12.22 (s, 1 H), 9.62 (d, J = 1.5 Hz, 1 H), 7.7–6.8 (m, 3 H), 4.06 (dd, J = 11.5 and 1.5 Hz, 1 H), 3.98 (d, J = 4 Hz, 2 H), 1.67, 1.51, and 0.99 (s, 3 H each).

In an earlier publication, the C-5 stereochemistry of this aldehyde was incorrectly assigned.[17] The C-11a, C-5 trideuterated aldehyde **33,** mp 167–171 °C, was prepared in the same way using deuterium oxide instead of water. Of the methine–methylene NMR signals, only a broad singlet remained at δ 2.42 (1 H), and the sharp doublet at δ 9.62 collapsed to a singlet. The IR spectrum showed small changes only in the fingerprint region.

Conversion of Aldehyde 32 into Aldehyde 33. The aldehyde **32** (0.5 g, 1.3 mmol) was dissolved in 1 N potassium hydroxide (10 mL). After

15 min at room temperature, the solution was acidified to pH 2 and extracted with ether (50 mL). The ether layer was dried and evaporated to yield a brown oil. The oil was taken up in ether (ca. 3 mL) and scratched to induce crystallization. The aldehyde **33** resulted, 255 mg (51%), mp 165–170 °C.

Enamine 34. A mixture of aldehydes **32** and **33,** melting range 120–160 °C (8.7 g, 0.033 mol), was dissolved in dry benzene (125 mL). Piperidine (2.8 g, distilled from potassium hydroxide) was added and the red solution was refluxed under a water separator for 1 h. The solvents were then removed to yield a brown, crystalline residue. The product was recrystallized from small amounts of ether, four crops yielding 9.7 g (91%) of yellow prisms of **34,** mp 114–118 °C, which became brown at room temperature after several days. The analytical sample was recrystallized twice from ether: mp 118–119 °C;[28] IR ($CHCl_3$) 1675, 1640 cm^{-1}; UV (CH_3OH) λ_{max} 217 nm (ϵ 23 700), 256 (12 600), and 338 (4200); NMR ($CDCl_3$) δ 12.23 (broad, 1 H), 7.6–6.7 (m, 3 H), 5.01 (d, J = 1.7 Hz, 1 H), 3.2–2.8 (m, 3 H), 1.61 (s, 3 H), 1.6–1.4 (m, 9 H), 1.02 (s, 3 H).

Hydrolysis of Enamine 34 to 33. The enamine **34** (0.6 g, 1.67 mmol) was dissolved in acetic acid (5 mL). Water (5 mL) was added and the solution was stirred for 2 h at room temperature. More water was then added and the product extracted with ether. The ether layer was washed with water, dried over magnesium sulfate, and evaporated to yield a light yellow oil. The oil crystallized upon addition of ether to yield 0.33 g of **33** (65%), mp 169–172 °C.

Cleavage of 34 on Silica Gel. The enamine **34** (0.3 g, 0.9 mmol) was dissolved in benzene (30 mL). Deactivated silica gel (20 g, Grace No. 950, stirred with water for 30 min, filtered, and air dried) was added with enough benzene to make a slurry. The mixture was allowed to stand for 3 h with occasional stirring. The original bright red color had faded completely at this time. The silica gel was extracted with ether, and the ether evaporated to yield a colorless oil. Addition of a little ether caused **33** to crystallize, 0.175 g (72%), mp 168–171 °C.

Alkylation of Enamine 34 (Preparation of 35). The enamine **34** (25.2 g, 0.077 mol) was dissolved in dry tetrahydrofuran and the system cooled to 0 °C. Sodium hydride (1.77 g, washed free of mineral oil with dry hexane and weighed under nitrogen) was added and the mixture was stirred slowly. When the bubbling of hydrogen was over (about 30 min) chloromethyl methyl ether (5.75 g, 0.077 mol) was added dropwise over 1.5 h, under nitrogen while stirring. After a total of 6 h stirring at room temperature, solvent was evaporated at 35 °C. The yellow residue was taken up in ether, and the precipitated sodium chloride filtered off. The ether was evaporated, and the orange oil crystallized from hexane, three crops, 25.5 g (90%) of **35,** pale yellow needles, mp 81–84 °C. Recrystallization from ether–hexane gave analytical material: mp 83–85 °C;[28] IR ($CHCl_3$) 1680, 1590 cm^{-1}; UV (methanol) λ_{max} 213 nm (ϵ 28 400), 251 (13 400), and 313 (3900); NMR ($CDCl_3$) δ 7.5–6.9 (m, 3 H), 5.23 (s, 2 H), 5.13 (d, J = 1.5 Hz, 1 H), 3.52 (s, 3 H), 3.2–2.8 (m, 7 H), 1.64 (s, 3 H), 1.6–1.3 (m, 9 H), 1.01 (s, 3 H).

Acetic Acid Hydrolysis of 35. The hydrolysis of **35** was carried out as described previously for **34.** Thus, **35** yielded a yellow oil from which crystallized 0.15 g (67%) of **33** upon addition of ether.

Silica Gel Cleavage of 35 (Preparation of Aldehyde 14). Exactly the same procedure was used as already described for the cleavage of **34.** Thus, **35** (3.0 g, 0.077 mol) on deactivated silica gel (150 g) yielded 2.5 g of **14** as a colorless oil which did not crystallize. The aldehyde absorption at 1730 cm^{-1} was nearly as strong as the tetralone carbonyl absorption at 1680 cm^{-1}. The UV spectrum had maxima at 312 and 252 nm. The NMR spectrum had an aldehyde proton at δ 9.59 and the C-5 proton at δ 4.11 as a pair of poorly resolved doublets, J = 11.5 and about 1 Hz. The methoxymethylene protons appeared at δ 5.21 and 3.48 as singlets. The integration suggested 70–80% of aldehyde **14** present in the crude oil.

Conversion of 14 into Oxazolone 17. The crude aldehyde **14** (1 g) and the oxazolinone **15**[30] (1.2 g) were dissolved in dry THF (60 mL) and treated with lead acetate trihydrate (0.75 g) at reflux for 1 h. Benzene (50 mL) was added. The usual lead salts were removed by filtration, and evaporation of solvents yielded an orange oil which crystallized upon addition of ether to give **17,** 0.76 g (40% over two steps from enamine **35**) of white clumps of small needles, mp 170–176 °C. The analytical sample was recrystallized from ether–chloroform, mp 180–84 °C dec.[28]

Conversion of 17 into Tetracyclic Product 19. A 50-mL, three-neck flask was fitted with a dropping funnel and a condenser, and was flushed with nitrogen. Sodium hydride in oil was washed several times with dry hexane prior to use, stored, and weighed under nitrogen.

Into the dropping funnel were placed the *N*-isopentenyl 3-oxoglutaramate **18**[30] (0.237 g) and sodium hydride (0.025 g). Dry THF (5 mL) was added to the funnel and the mixture was shaken until a homogeneous solution had been achieved. In the meanwhile, oxazolone **17** (0.60 g) was placed into the three-neck flask, dry THF was added (15 mL), and the solution was stirred under nitrogen. Sodium hydride (0.023 g) was suspended in a pan assembly within the three-neck flask such that it could be dropped into the reaction mixture when desired. The sodium salt of **18** was then added dropwise over 30 min while stirring for a total of 1 h. The initial red color faded to a pale yellow. At this time, the 0.023 g of sodium hydride was tripped into the reaction mixture and stirred until homogeneous. The red solution was then heated at 60 °C for 7 h. The red-brown reaction mixture was taken up in chloroform (300 mL) and washed rapidly with dilute hydrochloric acid (50 mL, pH 2). The chloroform was dried over sodium sulfate and evaporated to yield a brown oil.

The reaction product was chromatographed on 70 g of silica gel using chloroform (1% ethanol). A fast-moving, yellow band descended, and yielded 0.23 g of crude **19.** Subsequent fractions had no significant absorption above 320 nm, and yielded only orange, amorphous solids which were not characterized.

A portion of the product was chromatographed a second time to give **19** as a pale yellow, ether-soluble oil which could not be crystallized: λ_{max} (0.01 N HCl–CH_3OH), 262, 324 nm, relative peak heights 8:5; (0.1 N $Na_2B_4O_7$–MeOH, 1.5 h equilibration) 254, 340, and 450 nm, relative peak heights 2.5:1.8:1, respectively.

Hydrolysis of 19 to 36. A solution of **19** after one chromatography (0.105 g) in acetic acid (3 mL) was carefully diluted with water to the point of precipitating. The solution was then heated on the steam bath for 20 min. Colorless clumps of needles began to appear. The solution was allowed to cool while scratching. After 1 h at room temperature, 0.06 g (20% over two steps, based on oxazolone **17**) of **36** was collected, pale yellow, crystalline solid, decomposed above 240 °C. The analytical sample was recrystallized from ethyl acetate:[28] colorless needles, mp 258 °C dec; λ_{max} (0.01 N HCl–MeOH) 213 nm (ϵ 51 700), 263 (38 600), 324 (25 000); (0.1 M $Na_2B_4O_4$–MeOH) 212 (41 800), 253 (21 400), 438 (37 400), λ_{min} 355 (4000) after 1.5 h equilibration.

Cleavage of 36 to Nitrophthalide and Amine 37. A solution of **36** (0.05 g, crude after one chromatography) in 4:1 acetic acid–water (5 mL) was heated for 1.5 h at 100 °C. The solvent was evaporated under vacuum, and the product was filtered over neutral silica gel with ether. The first fractions (pale brown zone) crystallized to give 0.009 g of 3-nitrophthalide (82%). Further elution with ether produced traces of uncharacterized material (0.005 g). The column was then extracted with acetone, the acetone evaporated, and the residue extracted with chloroform to yield 0.013 g of amorphous solid, highly fluorescent in solution, λ_{max} in methanolic sodium borate at 434 nm, relative absorption at 250:434 nm = 2. The product failed to crystallize and was not investigated further.

Sequence from Unnatural Stereoisomer 32 to Tetracyclic 41b. A solution of **32** (1.45 g) in 100 mL of ether was combined with triethylamine (0.1 g), 2-phenylthiazolinone **16**[31] (1 g), and anhydrous $MgSO_4$ (3 g). After stirring for 2 h at 20 °C, the mixture was filtered and evaporated to give a brown oil. Filtration chromatography over Florisil (70 g) with $CHCl_3$ gave a fast-moving zone (250-mL fraction) containing 1.5 g of pale brown oil after evaporation of solvent. Addition of ether gave 0.66 g of crystalline thiazolone condensation product. After two recrystallizations from ether, an analytical sample was obtained, mp 170–175 °C dec.[28]

A solution of the thiazolone (0.224 g) from above and methyl *N-tert*-butyl-3-oxoglutaramate (**11**) was stirred with a catalytic amount of triethylamine (0.02 g) in benzene (20 mL) to induce Michael addition. After 2 h at 20 °C, the benzene was evaporated. The residue was dissolved in dimethylformamide (20 mL) and 0.224 g of NaH (hexane washed) was added. The flask was flushed with nitrogen and heated to 110 °C for 1.5 h. After cooling, the mixture was dissolved in chloroform (100 mL) and washed with 5% acetic acid. Removal of solvents gave a dark oil which was filtered over silica gel with chloroform. A fluorescent zone was collected which gave 0.285 g of brown, amorphous solid. Crystallization from ether gave **41b** (0.084 g, 26%) as yellow needles: mp 185–195 °C dec;[28] λ_{max} (0.01 M sodium borate in methanol) 493 nm (ϵ 29 000), 465 (38 000), 440 (27 000, infl).

Thiazolone 20. Aldehyde **14** was prepared as described earlier,

starting from 10 g of enamine **35,** and was used without further purification. The aldehyde was dissolved in 100 mL of dry THF. A fresh solution of 2-phenylthiazolinone **16** was then prepared by stirring dicyclohexylcarbodiimide (5.19 g, 25.2 mmol) and thiohippuric acid (4.9 g, 25.2 mmol) in 150 mL of THF (1 h, 20 °C).[31] The resulting slurry of **16** and dicyclohexylurea was then divided into two equal portions. Basic lead acetate (3.8 g, assumed to be $Pb_2OH(OAc)_3$, Baker & Adamson reagent) was added to the solution of aldehyde **14** and the first portion of 2-phenylthiazolinone **16** was added over several minutes to the vigorously stirred aldehyde-lead acetate mixture. After 30 min, the second portion of **16** was added and the reaction was allowed to proceed for a total of 1 h at 20 °C. The heterogeneous mixture was filtered through a sintered glass funnel using Celite to prevent clogging of the funnel pores. The filtrate was evaporated under reduced pressure, dissolved in 50 mL of chloroform, and slurried with 40 g of PF_{254} silica gel. The slurry was filtered using a large sintered glass funnel and washed with chloroform and ether. The combined filtrate was concentrated and crystallized from ether to give 9.5 g (77%) of thiazolone **20,** mp 152 °C. Recrystallization from ether gave analytically pure **20,** mp 157–160 °C dec.[28]

Preparation of Methyl 3-Oxoglutaramate 21. Dimethyl 3-oxoglutarate (160 g, 0.92 mol) was dissolved in 1 L of methanol. This solution was saturated with ammonia at 0 °C and then let stand at room temperature for 10 h. After an additional 12 h at 5 °C, the solution was evaporated under vacuum to a total volume of about 200 mL. Ethyl acetate (750 mL) was added to this concentrate while stirring vigorously. The precipitate was removed by filtering the entire solution through 80 g of silica gel (60–200 mesh, grade no. 950, Fisher). The filtrate was concentrated under vacuum and diluted with a few milliliters of methanol. Filtration after 5 h at 0 °C yielded 27.5 g of white crystals of **38.** The mother liquor was concentrated, diluted with 250 mL of methanol, and allowed to crystallize at − 20 °C. Enamine (20 g) was obtained as a second crop. Total yield of **38** was 47.5 g (33%): mp 120–121 °C from methanol;[28] NMR (Me_2SO-d_6–$CDCl_3$ (v/v) 40:60) δ 6.5–7.7 (4 H, broad, rapid exchange with D_2O), 4.55 (1 H, singlet), 3.62 (3 H, singlet), 3.05 (2 H, singlet).

Hydrolysis of 22 g (0.139 mol) of recrystallized enamine **38** (in 360 mL of chloroform) proceeded rapidly upon addition of 12 mL of concentrated HCl (vigorous stirring). Magnesium sulfate (anhydrous) (20 g) was then added after hydrolysis was complete. The solution was filtered and concentrated under vacuum. Crystallization from ethyl acetate yielded 13.2 g (60%) of white crystals of **21,** mp 36–38 °C.[28] The product required exclusion of moisture for storage: UV λ_{max} (CH_3OH) 243 nm (ϵ 2510), λ_{max} (NaOH–CH_3OH) 273 (17 500); NMR (Me_2SO-d_6–$CDCl_3$ (v/v) 40:60) δ 6.5–7.7 (2 H, broad, rapid exchange with D_2O), 3.7 (5 H, overlapping singlets), 3.5 (2 H, singlet), and several minor peaks perhaps due to an enolic form.

Thiazolone Condensation to Tetracycle 22. Isolation of 39a, 40, and 40a. Methyl 3-oxoglutaramate **21** (1.59 g, 10.0 mmol) in 40 mL of tetrahydrofuran was treated at −78 °C under nitrogen atmosphere and vigorous stirring with 4.16 mL (10 mmol) of butyllithium solution (2.4 M in hexane). The mixture was stirred for 10 min, by which time a bright yellow, homogeneous solution resulted. A solution of 4.72 g (9.55 mmol) of thiazolone **20** in 90 mL of tetrahydrofuran was added dropwise over 30 min to the lithio glutaramate salt solution maintained at −78 °C. The cooling bath was removed, and the mixture was allowed to warm to 25 °C over 1.5 h and then refluxed for 1 h. The mixture was cooled to −78 °C before 0.5 mL of *tert*-butyl alcohol was added (dried over and distilled from calcium hydride) and then butyllithium (11.4 mmol, 4.75 mL of 2.4 M hexane solution). The cold, green solution was warmed to room temperature over 30 min, treated with 2.1 mL of lithium *tert*-butoxide (0.61 M in tetrahydrofuran), stirred at 50–60 °C for 1 h, and then refluxed for 2 h. During the 2-h reflux period, additional portions of base were added to the deep wine red colored reaction mixture (2.8 and 7.2 mL of lithium *tert*-butoxide solution after 50 and 90 min, respectively). The mixture was cooled to 0 °C, acidified with 3 mL of acetic acid while stirring, concentrated in vacuo at 25 °C, and distributed between 250 mL of benzene–ethyl acetate (1:1) and a 100-mL portion of ice water. The organic phase was washed with 100 mL of ice water, concentrated (aspirator), and evaporated twice with benzene (200 mL) and $CHCl_3$ (30 mL) to remove traces of water. The brown, oily residue was dissolved in 15 mL of chloroform and 5 mL of tetrahydrofuran and diluted with ether to cloudiness. Crystallization at −12 °C overnight afforded 609 mg of chromatographically pure **22.** A second crop of 409 mg was obtained from the mother liquor. Chromatography of material remaining in the mother liquors on 100 g of polyamide (deactivated with 20 mL of benzene) using benzene–chloroform–acetic acid (50:50:1) as eluent afforded in fractions 5–16 (30 mL each) an additional 352 mg. The total yield of **22** was 1.370 g or 27%. A 24% yield was obtained on repeating the experiment.

The material in chromatography fractions 1–4 of these two experiments was combined, dissolved in 150 mL of acetic acid and 15 mL of water, and heated to reflux temperature over 2 min. The boiling solution was poured onto 200 g of ice, taken up in 3 × 400 mL of chloroform, and washed three times with 400-mL portions of water. Polyamide (40 g) (deactivated with 8 mL of eluent) was used to chromatograph the residue from evaporation of the organic phase. With benzene–hexane–acetic acid (100:100:1) as eluent, fractions 4–8 yielded 217 mg of **39a** after evaporation and crystallization from ethanol. Fractions 13 and 14 yielded 72 mg of **40** from toluene. Fractions 9–11 contained nontetracyclic impurities, some **40,** and a little **40a** which possesses a slightly greater R_f value on TLC plates than **40.** Repeated chromatography on polyamide with benzene–carbon tetrachloride–acetic acid (100:100:1) as eluent and fractional crystallization from ethanol–chloroform gave pure crystalline **40a.**

Tricycle 39a: mp 221–223 °C after three recrystallizations from ethanol–tetrahydrofuran;[28] IR (KBr) 5.85 μ; UV (0.01 N HCl–CH_3OH, 14-h equilibration) λ_{max} 330 nm (ϵ 4270), 260 (33 900), (0.01 N NaOH–CH_3OH) λ_{max} 330 (3960), 258 (26 700), 233 sh (20 400); NMR (Me_2SO-d_6) 0.65 s (CH_3), 1.16 s (CH_3), 1.57 s (CH_3), 2.31 d (H_{5a}, J = 12 Hz), 4.07 dd (H_5, J = 2, 12 Hz), 2.80 s (broad, H_{11a}), 3.15 d (broad, H_{11a}, J = 5 Hz), 3.46 t (H_{4a}, broad, J = 5 Hz), 3.77 s (CO_2CH_3), 4.02 (d (H_{12a}, J = 5 Hz), 5.77 dd (J = 5, 8 Hz, H_4), 6.65–7.7 m (8 H, ArH), 8.90 and 9.17 s (NH_2), 10.26 d (NHCS, J = 8 Hz), 12.20 s (ArOH), 16.15 s (OH).

Tetracycle 22: mp 225 °C dec after recrystallization from acetone;[28] UV (0.1 N sodium borate–MeOH) 452 nm (ϵ 9870), 337 (20 000), 250 (26 700); (0.01 N HCl–MeO) 315 (22 100), 262 (30 000); NMR, Table II.

Tetracycle 40: mp 220 °C dec, recrystallized from $CHCl_3$;[28] UV (0.1 N sodium borate–MeOH) 440 nm (ϵ 34 300), 310 (11 000), 240 (20 900), (0.01 N HCl–MeOH) 315 (24 700), 257 (32 900); NMR, Table II.

Tetracycle 40a (4-Epi Series): mp 207 °C dec, fine yellow needles from chloroform–ethanol; IR (KBr) 6.14 (s, 6.20 (s), 6.48 (s) μ; UV (0.1 N sodium borate–MeOH) λ_{max} 443 nm (ϵ 35 000), 300 (11 700), 235 (sh, 19 200); (0.01 N HCl–MeOH) 312 (21 500), 262 (26 300), 215 (20 900); (0.01 N NaOH–MeOH) 427 (15 900), 395 (sh, 11 600), 375 (sh, 10 200), 238 (sh, 15 100), 255 (sh, 19 400), 235 (sh, 20 900); NMR, Table II.

Cyclization of Tricycle 39a to Tetracycle 40b. Tricycle **39a** (700 mg, 1.15 mmol) was suspended in 50 mL of absolute *tert*-butyl alcohol. The suspension was stirred vigorously under nitrogen while adding first 4.8 mL (11.5 mmol) of butyllithium solution (2.4 M in hexane) and then 10 mL of absolute *N,N*-dimethylformamide. The reaction mixture remained partially inhomogeneous during a reflux period of 6 h. After the mixture had cooled to room temperature, 7 mL of acetic acid in 13 mL of water was added before the mixture was poured into 200 mL of ethyl acetate and 200 mL of water. After extraction of each phase with two more portions of the respective solvent, the organic phases were concentrated to a bright yellow oil which was chromatographed on 20 g of polyamide (pretreated with 2 mL of eluent) using benzene–carbon tetrachloride–methanol (50:50:1) as eluent. Fractions 1–3 (30 mL each) yielded 213 mg of recovered **39a** (30%, recrystallized from ethanol) and fractions 4–7 gave 259 mg (39%) of 4a-epi tetracycle **40b,** mp 215 °C dec, recrystallized from chloroform–ethanol as golden yellow needles,[28] golden cubic crystals containing 1 mol of water from acetone–ethanol–water: IR (KBr) 6.12 (s), 6.45 (s) μ; UV (in sodium borate–MeOH) λ_{max} 436 nm (ϵ 24 300), 393 (12 600, sh), 370 (10 100, sh), 305 (10 900, sh), 243 (25 300) (equilibrated for 70 min); (in 0.01 N HCl–MeOH) λ_{max} 312 (19 500), 262 (27 500), 215 (24 200); (in 0.01 N NaOH–MeOH) 386 (sh, 13 200), 368 (19 400), 358 (17 600, sh), 248 (27 700); NMR, Table II.

Hydrolysis of 22 to 40. Cyclization product **22** (2.4 g, 3.9 mmol) was added to a 500-mL round-bottom flask containing 200 mL of acetic acid and 20 mL of water. This mixture was stirred and heated rapidly over a 6-min period to reflux temperature at which time the solution had become homogeneous. After refluxing for 2 min, the solution was cooled rapidly in cold water and then concentrated under reduced pressure. The solution did not exceed 30 °C during evaporation. Crystallization from chloroform yielded 2.0 g (90%) of **40** (mp

220 °C dec). TLC on polyamide (carbon tetrachloride–benzene–formic acid, 100:100:1) showed no evidence for epimerization at C-4 during hydrolysis.

Hydroxylation of 40. To a solution of tetracycle **40** (524 mg, 0.91 mmol) in 50 mL of tetrahydrofuran and 35 mL of absolute dimethylformamide were added 260 μL of triethyl phosphite and 40 μL of water. This mixture was rapidly added to a vigorously stirred suspension of sodium hydride in mineral oil (350 mg of 50% mineral oil suspension, 7.3 mmol of NaH). A strong stream of oxygen was bubbled through the reaction mixture immediately after combination of the reactants. (*Caution! Explosions occur unless the gas dispersion tube is completely immersed in the solution before oxygen is introduced.* An explosive mixture of solvent vapor, hydrogen gas, and oxygen is present which can be ignited by traces of noble metal catalyst often present in the dispersion tubes.) A 40-μL portion of water was injected into the mixture immediately after combination of reactants and another 40 μL of water was added after 1 min. After 13 min, the oxygen stream was replaced by a stream of dry nitrogen and the deoxygenated solution was treated with a solution of 2 mL of acetic acid in 6 mL of water to destroy excess sodium hydride. This mixture was worked up by distributing five times between 200 mL of ethyl acetate and 200 mL of ice water. Freed of inorganic salts, the organic phases were concentrated in vacuo. The residue was dissolved in 20 mL of 0.01 N methanolic hydrochloric acid and let stand at 25 °C for 1.5 h. Methanol (50 mL) was added and mineral oil was extracted with hexane (50 mL). The methanol phase was diluted with 200 mL of water and extracted three times with 200-mL portions of ethyl acetate. Chromatography of the concentrated organic phase on 10 g of polyamide (pretreated with 2 mL of chloroform plus 0.2 mL of methanol) employing chloroform–acetone–methanol (100:10:1) as eluent afforded in the first fraction (30-mL fractions) 74 mg of unchanged starting material (14%, crystallized from toluene). Fractions 2–5 yielded 65 mg (12%) of **44** on crystallization from benzene and 236 mg (47%, crystallized from ethyl acetate–methylene chloride) of **43** from fractions 6–12.

11a-Hydroxylated Tetracycle 44: mp 220 °C dec, recrystallized three times from acetone–methanol;[28] IR (KBr) 6.22 (s), 6.55 (s) μ; UV (0.01 N HCl–MeOH) λ_{max} 317 nm (ε 20 000), 260 (31 500); (0.01 N NaOH–MeOH) 348 (17 700), 255 (28 300); NMR, Table II.

12a-Hydroxylated Tetracycle 43: mp 200 °C dec, from ethyl acetate, containing one molecule of solvent by NMR;[28] UV (0.01 N NaOH–MeOH) 373 nm (ε 17 600), 246 (28 900); (0.01 N HCl–MeOH) 360 (15 400), 267 (27 400); NMR, Table II.

Amide Cleavage. Amine Hydrochloride 47. Thioamide **43** (206 mg, 0.32 mmol) was stirred under nitrogen atmosphere at 25 °C for 20 h with 0.6 mL (9.6 mmol) of methyl iodide in 3 mL of tetrahydrofuran. Volatile solvent and reagent were removed in vacuo at 25 °C, and the crude product was hydrolyzed in a solution of 2 mL of 0.5 N hydrochloric acid and 4 mL of tetrahydrofuran during 1.5 h at 25 °C. The hydrolysate was diluted with 8 mL of water and washed once with 10 mL of ether. The aqueous phase was extracted with 5- and 10-mL portions of ethyl acetate and then with two 5-mL portions of 1-butanol. The butanol extracts were evaporated at 25 °C under high vacuum to an oil from which crystallized amine hydrochloride **47** (yellow powder, 123 mg) on addition of a little ethyl acetate. The product contained considerable anhydro material **45** (absorption at 420 nm) which was probably formed during concentration of the acidic butanol extracts. No attempt was made to purify **47,** and crude material could be used in the next step. A second product (55 mg) was recovered from the ether–ethyl acetate extracts by crystallization from acetone–DMF, yellow needles, mp 225 °C dec. This substance analyzed correctly for the amide derived from **43** by exchange of oxygen for sulfur:[28] UV (0.01 N NaOH–MeOH) λ_{max} 370 nm (ε 17 600), 265 (23 400), 245 (27 200) (16-h equilibration); (0.01 N HCl–MeOH) λ_{max} 365, 267, 225 nm (34:77:87) (extremely insoluble, qualitative spectrum); IR (KBr) 6.18, 6.31, 6 48 μ.

Alkylation of Amine Hydrochloride. *dl*-Terramycin. Amine hydrochloride **47** (166 mg, 0.35 mmol, impure yellow powder) was stirred with 5 mL of tetrahydrofuran, 40 μL of ethanol, 122 μL (0.71 mmol) of *N,N*-diisopropylethylamine, and 180 μL (1.9 mmol) of dimethyl sulfate for 17 h at 25 °C. The mixture was inhomogeneous at first but gradually became a golden yellow, homogeneous solution toward the end of the reaction. The mixture was evaporated in vacuo at 25 °C to a dry residue and chromatographed on 6 g of polyamide (deactivated with 0.5 mL of methylene chloride and 50 μL of methanol). Chloroform eluted racemic, synthetic terramycin in fractions 5–8 (20 mL each). After crystallization from methylene chloride and a little acetone, 60 mg of *dl*-terramycin was obtained, 23% overall from thioamide **47.** NMR, UV, and microanalysis data indicated that 0.8 mol of acetone was present in the dry, crystalline product, mp 200 °C.[28]

The infrared spectrum (KBr) was similar to but not identical with that of authentic terramycin. A spectrum using tetrahydrofuran as solvent showed the presence of acetone. A molecular ion at *m/e* 460 appeared in the mass spectrum. The fragmentation pattern was quite similar to that of authentic terramycin, although both samples degraded thermally in the probe. The NMR spectra of racemic and natural terramycin in pyridine-d_5 were identical, provided that natural terramycin recrystallized from acetone was used. Ultraviolet spectra in acid and base of the two samples were superimposable. Quantitative ultraviolet spectroscopy gave for racemic terramycin λ_{max} (NaOH–CH_3OH) 375 nm (ε 16 800), 265 (17 800), 247 (18 600); λ_{max} (HCl–CH_3OH) 360 (14 300), 270 (19 300).

Synthetic and authentic samples showed identical behavior on polyamide TLC using the solvent systems acetic acid–chloroform (2:98), acetic acid–chloroform–acetone (1:89:10), and acetic acid–methanol–benzene (10:5:85). A biological assay was also performed by Chas. Pfizer and Co., Inc.[26] This assay showed synthetic (±)-terramycin to be almost exactly 50% as active as authentic terramycin.

Supplementary Material Available: The NMR spectra of **40** under a variety of conditions (4 pages). Ordering information is given on any current masthead page.

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(32) We are grateful to Dr. I. A. Solomons and Dr. L. H. Conover of Chas. Pfizer Medical Research Laboratories for supplying us with authentic terramycin.

Biosynthesis of the Antibiotic Granaticin

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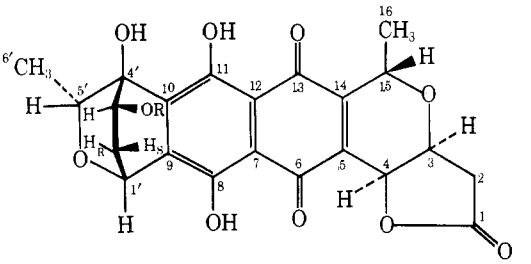
Abstract: The antibiotic granaticin (Ia) is synthesized by *Streptomyces violaceoruber* from eight acetate units, which are assembled into a benzoisochromane quinone moiety, and a molecule of glucose, which is converted into a 2,6-dideoxyhexose and attached to the aromatic moiety by carbon–carbon linkages at C-1 and C-4. Conversion of glucose into the 2,6-dideoxyhexose moiety proceeds with retention of H-1, H-2, H-4, and the hydrogens at C-6 and loss of H-3 and H-5. The hydroxyl group at C-6 of glucose is replaced with inversion of configuration by a hydrogen which is transferred intramolecularly from C-4, indicating operation of the dTDP-glucose oxidoreductase reaction as the first pathway-specific step. The hydroxyl group at C-2 of the hexose is replaced by hydrogen with retention of configuration. The last step in the biosynthesis of granaticin seems to be formation of the five-membered lactone ring; a cell-free extract of *S. violaceoruber* was shown to catalyze formation of granaticin from dihydrogranaticin (IIa) without incorporation of ^{18}O from $^{18}O_2$.

Introduction

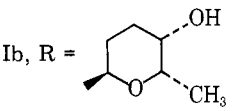
The antibiotic granaticin (Ia)[1] was first isolated from *Streptomyces olivaceus*,[2] but was also detected in a number of other actinomycetes, e.g., in *S. violaceoruber*, in which it co-occurs with the glycoside granaticin B (Ib),[3] and in *S. litmogenes*.[4] The structures of Ia and Ib were established by a combination of chemical degradations[5] and an X-ray analysis.[6] Granaticin B is the α-L-rhodinoside of granaticin. Granaticin belongs to a class of microbial metabolites which is characterized by the presence of a benzoisochromane quinone system and which also includes kalafungin,[7] frenolicin,[8] actinorhodin,[9] the nanaomycins,[10,11] the naphthocyclinones,[12,13] and the griseusins.[14] Recently, three cometabolites of granaticin were isolated and their structures were assigned as dihydrogranaticin (IIa) and the anthraquinones IIIa and IIIb.[15]

Granaticin is active against Gram-positive bacteria and protozoa, but has little or no activity against Gram-negative bacteria, mycobacteria, fungi, or yeasts.[16,17] It has some activity against P-388 leukemia[4] and granaticin B inhibits various transplanted tumors in rodents (cf. footnote 1 in ref 18). Granaticin inhibits RNA synthesis and to a lesser extent DNA and protein synthesis.[19] The antibiotic blocks the charging of Leu-tRNA by inhibiting leucyl-tRNA synthetase;[19] it also inhibits RNA-dependent DNA synthesis, but by an interaction with the template rather than with the enzyme, reverse transcriptase.[18]

In the present communication we report results which establish the overall biosynthetic origin of granaticin and which provide detailed information on certain aspects of its mode of formation.



Ia, R = H, granaticin



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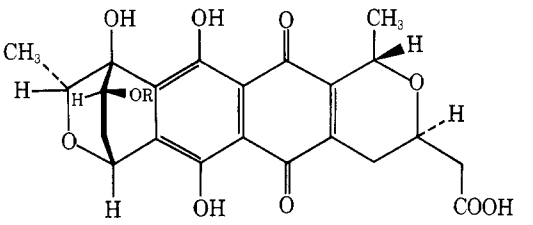
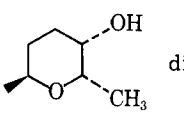


IIa, R = H, dihydrogranaticin



IIb, R = dihydrogranaticin B

Results

The biosynthesis of granaticin was studied in shake cultures of *S. violaceoruber* strain Tü 22,[3] which were grown in complex media based on peanut meal/glucose (acetate feeding experiments) or malt extract/yeast extract/glucose (cell-free experiments) or in a synthetic medium containing mannose

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