metabolized. Experiments are now being carried out in order to establish the biological role of the $\Delta^{8,14}$ sterol dienes.

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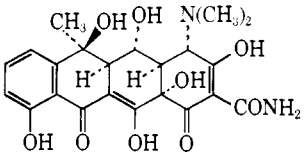
Tetracyclines. VII. Total Synthesis of *dl*-Terramycin[1]

Sir:

Terramycin is one of the most important broad-spectrum antibiotics used in medicine today. It was the first member within the family of tetracycline antibiotics to have its structure fully elucidated in the laboratories of Chas. Pfizer & Co., Inc., in close cooperation with Woodward.[2] Structure and configuration **1** for this compound have been confirmed by X-ray analysis[3,4] and by nmr analysis.[5]



1

Because Terramycin (**1**) is one of the most highly substituted and chemically labile members of the tetracycline family, its synthesis has remained an intriguing and challenging problem. We now wish to report the first synthesis of this compound as its racemate.[6,7] This synthesis is another example of a general method[8] for synthesizing tetracyclines of both known and novel structures.

(1) Terramycin is a registered trademark of Chas. Pfizer & Co., Inc. for oxytetracycline.

(2) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Ragna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, 75, 5455 (1953).

(3) Y. Takeuchi, and M. J. Buerger, *Proc. Natl. Acad. Sci. U. S.*, 46, 1366 (1960).

(4) H. Cid-Dresdner, *Z. Kristallogr.*, 121, 170 (1965).

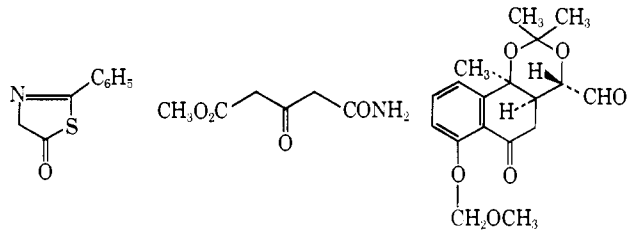
(5) M. Schach von Wittenau, R. K. Blackwood, L. H. Conover, R. H. Glauert, and R. B. Woodward, *J. Am. Chem. Soc.*, 87, 134 (1965).

(6) The simplest compound deriving from a fermentation product and having full antibacterial activity is 6-demethyl-6-deoxytetracycline. This compound was first synthesized by J. J. Korst, J. D. Johnston, K. Butler, E. J. Bianco, L. H. Conover, and R. B. Woodward, *ibid.*, 90, 439 (1968) [preliminary reports: (a) L. H. Conover, K. Butler, D. Johnston, J. J. Korst, and R. B. Woodward, *ibid.*, 84, 3222 (1962); (b) R. B. Woodward, *Pure Appl. Chem.*, 6, 561 (1963)]. Another synthesis of this compound was reported later.[8]

(7) A synthesis of 12a-deoxy-5a,6-anhydrotetracycline has recently been reported: A. I. Gurevich, M. G. Karapetyan, M. N. Kolosov, V. G. Korobko, S. A. Popravko, and M. M. Shemyakin, *Tetrahedron Letters*, 131 (1967). Syntheses of other tetracyclic compounds deriving from tetracyclines are summarized by H. Muxfeldt and R. Bangert, *Progr. Chem. Org. Nat. Products*, 21, 116 (1963).

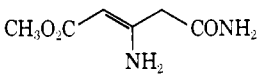
(8) H. Muxfeldt and W. Rogalski, *J. Am. Chem. Soc.*, 87, 933 (1965).

Terramycin (**1**) was assembled from three basic building blocks: the thiazolone **2**, methyl 3-oxoglutaramate (**3**), and the aldehyde **4**. The preparation of



2 **3** **4**

the thiazolone **2** has been described recently.[9] Methyl 3-oxoglutaramate (**3**) (mp 36–38°; λ_{max} mμ (ϵ) 273 (17,500) in 0.01 *N* NaOH; λ_{max} μ 5.75, 5.80, 5.95, and 6.30 in $CHCl_3$) was obtained by acid hydrolysis of the enamine **5** (mp 120–121°; λ_{max} mμ (ϵ) 276 (16,600) in MeOH; λ_{max} μ 5.96, 6.18, and 6.40 in $CHCl_3$). Enamine **5** was prepared by carefully controlled treatment of dimethyl 3-oxoglutarate with ammonia in methanol.



5

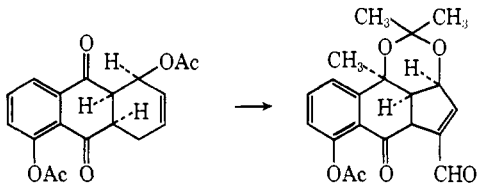
The synthesis of aldehyde **4** has already been published in part.[10] Starting material was the diene adduct **6** of juglone acetate and 1-acetoxybutadiene. This compound was converted over seven steps in high yield into the aldehyde **7**. Ozonolysis followed by hydrolysis of the crystalline ozonide yielded a crystalline mixture of **8** and **9**. Aqueous sodium carbonate cleaved these substances in 85% yield to a mixture of aldehydes **10** and **11** (melting range 120–160°). The pure isomers, mp 140–143° and 171–173°, respectively, could be obtained. That the higher melting aldehyde is aldehyde **11** was deduced from its nmr spectrum (H_{4a}, δ 9.60, d, J = 1.5 Hz; H_5, δ 4.06, dd, J = 1.5 and 11.5 Hz; H_{5a}, δ 2.43, dt, J = 11.5 and 4.0 Hz; 11a protons, δ 2.98, d, J = 4.0 Hz; in $CDCl_3$).[11] Cleavage of the mixture of **8** and **9** in deuterium oxide with sodium carbonate to aldehydes **12** and **13** further confirmed that in **11** and **13** epimerization at C-5 had occurred. Aldehyde **13** had incorporated deuterium at C-5 as evidenced by the nmr spectrum (H_{4a}, δ 9.60, s; H_{5a}, δ 2.42, s; in $CDCl_3$).

The desired aldehyde **4** could be easily prepared from the mixture of aldehydes **10** and **11** by a three-step procedure. Piperidine in refluxing benzene converted the aldehydes to **14** (91%; mp 118–119°; λ_{max} μ 5.97 and 6.10 in $CHCl_3$). This enamine was alkylated with chloromethyl methyl ether *via* its sodium salt to **15** (90%; mp 81–84°; λ_{max} μ 5.96 and 6.28 in $CHCl_3$). When **15** was adsorbed on deactivated silica gel, selective hydrolysis of the enamine function occurred, and the oily aldehyde **4** was formed (72%). This hydrolysis was stereospecific since **4** had an nmr spectrum consistent only with a *trans* coplanar relationship of the hydrogens at C-5 and C-5a (H_{5a}, δ 9.59, d, J = 1.0 Hz; H_5, δ 4.11, dd, poorly resolved, J = 1 and 11.5 Hz; in $CDCl_3$). Furthermore the aldehyde **11** was regenerated

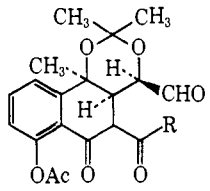
(9) H. Muxfeldt, J. Behling, G. Grethe, and W. Rogalski, *ibid.*, 89, 4991 (1967).

(10) H. Muxfeldt, *Angew. Chem.*, 74, 825 (1962). The melting points of 10 and 11 are interconverted in this paper.

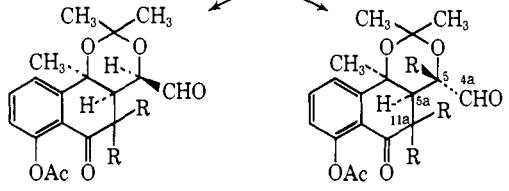
(11) Numbering as in Terramycin.



6 7



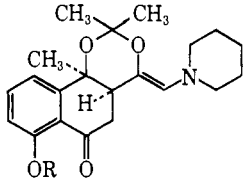
8, R = CHO
9, R = CO_2H



10, R = H
12, R = D

11, R = H
13, R = D

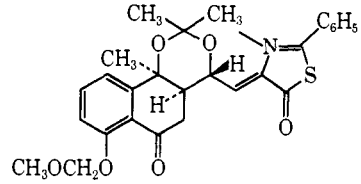
in almost quantitative yield by acetic acid treatment of **4**.



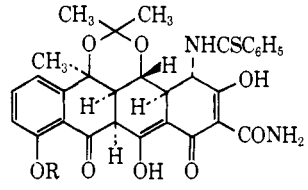
14, R = H
15, R = CH_2OCH_3

Condensation of **4** with thiazolone **2** in the presence of basic lead acetate in tetrahydrofuran (THF) gave thiazolone **16** (77%; mp 157–160°; H_{4a}, δ 6.47, d, J = 8.5 Hz; H_5, δ 5.33, dd, J = 8.5 and 11.0 Hz; in $CDCl_3$; λ_{max} mμ (ϵ) 205 (37,500), 257 (16,700), 279 (18,900), and 322 (11,700) in CH_3CN). The nmr spectrum of **16** indicated clearly that the configuration at C-5a was retained during the condensation process.

A combination of strong bases (butyllithium and potassium *t*-butoxide) catalyzed the reaction (THF, reflux) of thiazolone **16** with the lithium salt of methyl 3-oxoglutaramate (**3**) to give the tetracyclic compound **17** (21%; mp 225° dec; λ_{max} mμ (ϵ) 452 (9870), 337 (20,000), 250 (26,700) in 0.1 *M* MeOH–$Na_2B_4O_7$ after equilibration for 90 min; 315 (22,100), 262 (30,000) in 0.01 *N* MeOH–HCl after equilibration for 4 hr. The low solubility of this compound in appropriate solvents made nmr spectral data difficult to obtain. Removal of the methoxymethyl group (acetic acid) led to the more soluble **18** (90%; mp 220° dec; λ_{max} mμ (ϵ) 440 (34,300), 310 (11,000), 240 (20,900) in 0.1 *M* MeOH–$Na_2B_4O_7$ after equilibration for 45 min; 315 (24,700), 257 (32,900) in 0.01 *N* MeOH–HCl). Coupling constants, $J_{NH,H4}$ = 9 Hz, $J_{H4,H4a}$ = 12.5 Hz, $J_{H4a,H5}$ = 8 Hz, $J_{H5,H5a}$ = 11 Hz, and $J_{H5a,HIIa}$ = 5 Hz, from the nmr spectrum of **18** proved the configuration of this compound. In addition, these coupling constants were verified by decoupling on a 100-Mc instrument. Deuterium exchange made specific assignments in the spectrum of **18** even easier.

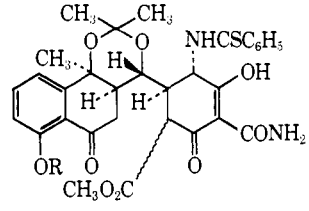


16



17, R = CH_2OCH_3
18, R = H

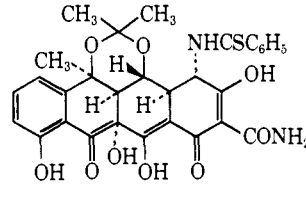
Evidence has been accumulated that **17** is formed *via* intermediates of type **19**, which can be prepared by reaction of **16** with the lithium or sodium salt of **3**. Compounds of type **19** and also of type **20** can be cyclized.



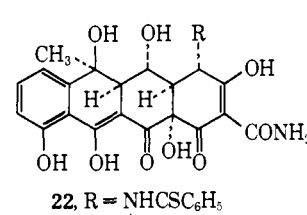
19, R = CH_2OCH_3
20, R = H

Hydroxylation of **18** in basic media with molecular oxygen led after acid hydrolysis (0.01 *N* MeOH–HCl, room temperature) to **22** (32% light yellow crystals; mp 200° dec; λ_{max} mμ (ϵ) 373 (17,600), 246 (28,900) in 0.01 *N* MeOH–NaOH; 360 (15,400), 267 (27,400) in 0.01 *N* MeOH–HCl). The absence of the acetonide group in **22** is obvious from the nmr spectrum. The virtual identity of the uv spectra of **22** with those of Terramycin (**1**) (if allowance is made for the thiobenzamide chromophore) is evidence for **22** having rings A and B *cis* fused.[12]

In the mother liquor of **22** another oxidation product was found. Although not fully characterized, this compound has been assigned structure **21** in view of uv and nmr spectral data.



21



22, R = $NHCSC_6H_5$
23, R = $\overset{+}{N}H{=}C(SCH_3)-C_6H_5\ I^-$
24, R = $\overset{+}{N}H_3\bar{C}l$

(12) L. H. Conover, Special Publication No. 5, The Chemical Society, London, 1956, p 48.

Treatment of **22** with methyl iodide (room temperature, THF) led to the thioimino ether **23**, which was not isolated but immediately hydrolyzed in dilute acid to give the crystalline hydrochloride of **24** (82%) contaminated with a small amount of an anhydro compound. This material was immediately alkylated (dimethyl sulfate, Hünig's base, THF). After purification by chromatography on polyamide, Terramycin (**1**) was isolated (33%) as light yellow crystals (mp 200° dec) containing 0.8 mol of acetone after thorough drying. Terramycin obtained by fermentation[13] also contained about 1 mol of acetone when recrystallized from acetone. Synthetic and authentic Terramycin samples were then compared. The nmr spectra in pyridine-d_5 were identical provided that comparison was made at the same concentration. Ultraviolet spectra were superimposable. The identity was further established by mass spectral data and chromatography on polyamide in different solvent systems.[14] A bacteriological assay[13] showed synthetic Terramycin to be 50% as active as Terramycin from *Streptomyces rimosus*. Elemental analyses of synthetic Terramycin (plus 0.8 mol of acetone as measured from the nmr spectrum) and of all crystalline intermediates are in agreement with the structures.

Acknowledgment. We are grateful to the National Institutes of Health (Grants E-4221, Al 04221-02 to -05, and Al 07663-01 and -02) and to the National Science Foundation for making this work and necessary model work for this project possible. We are also pleased to acknowledge generous financial aid by unrestricted research grants from Chas. Pfizer and Co., Inc., and from the Hoffmann-La Roche Foundation.

(13) 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. We also thank Mr. Roland Plude for running the bioassay of synthetic Terramycin

(14) Systems applied distinguished clearly between Terramycin, N-demethylterramycin, 4-epiterramycin, and **24**.

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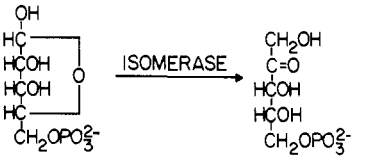
Received September 8, 1968

On the Structure of Ribulose 5-Phosphate as an Intermediate of the Photosynthetic Pentose Phosphate Cycle[1]

Sir:

The product of the oxidative decarboxylation of 6-phosphogluconate by the specific dehydrogenase was shown to be ribulose 5-phosphate (**2**). The same report[2] also described the discovery of ribose phosphate isomerase (isomerase) which was shown to catalyze the interconversion of **2** and ribose 5-phosphate (**1**). This communciation describes the results of absorption, rotatory dispersion, and circular dichroism studies of the spinach leaf chloroplast isomerase catalyzed reaction using **1** as the substrate. The results of these studies are not in accordance with the predicted spectral properties of **2** which, therefore, leads to the conclusion that structure **2** does not hold for the reductive pentose phosphate cycle.

Solutions of **1** in 0.037 *M* potassium phosphate (pH 7.38) are transparent above 220 mμ. The ultraviolet absorption spectrum of the isomerase-catalyzed reaction showed an absorption band with λ_{max} 280 mμ and a minimum at 242 mμ. The initial rate of the isomerase-catalyzed reaction, measured at a fixed wavelength of 280 mμ, varied linearly with isomerase concentration. The time course of the reaction was determined by direct spectrophotometric measurement at 280 mμ and by the colorimetric keto sugar assay[3] which is the conventional technique for the measurement of isomerase activity. The time course curves coincided when they were plotted as per cent reaction, 100% reaction being the optical density end points for



1 **2**

the spectrophotometric and colorimetric assays, 0.0231 at 280 mμ and 0.729 at 540 mμ, respectively.[4]

The addition of 0.10 ml of 1.0 *N* NaOH to 0.90 ml of the isomerase-generated chromophore caused a bathochromic shift in λ_{max} of 28.5 mμ.[5] Neutralization of the NaOH led to a reversal of the bathochromic shift, a hypsochromic shift of 28.5 mμ which regenerated the original chromophore. The addition of an excess of HCl, however, led to a hypsochromic shift of 33.5 mμ, from 308.5 to 275 mμ. The absorption band with λ_{max} 275 mμ was not stable, a spontaneous bathochromic shift of 5 mμ occurring in the acidic solution which regenerated the chromophore formed from **1** by isomerase. This 5-mμ bathochromic shift was characterized by an isoabsorption point at 296 mμ which showed that only two chromophores were involved in the spectral shift. The addition of 0.10 ml of 1.0 *N* HCl to 0.90 ml of the isomerase-generated chromophore left its absorption spectrum unchanged. A kinetic analysis of the NaOH-induced bathochromic shift showed the reaction to be first order with a rate constant, k, of approximately 1.9×10^{-3} sec^{-1}. The rate equation was $\log [1/(C - A_{308.5})] = kt$, where C is the ratio of the initial concentration of the isomerase-generated chromophore to the molar extinction co-

(1) This work was supported in part by National Science Foundation Grant GB-6795 and the Cancer Research Funds of the University of California.

(2) B. L. Horecker, P. Z. Smyrniotis, and J. E. Seegmiller, *J. Biol. Chem.*, **193**, 383 (1951).

(3) B. Axelrod and R. Jang, *ibid.*, **209**, 847 (1954). This determination is a modification of the procedure originally described by Z. Dische and E. Borenfreund, *ibid.*, **192**, 583 (1951).

(4) The initial concentration of **1** was 2.0×10^{-3} *M* for the correlation of the spectrophotometric and colorimetric assay procedures. The isomerase activity in the substrate solution was approximately 0.014 μmol of **2**/min as assayed by the colorimetric assay.

(5) Axelrod and Jang observed that **2** in 0.1 *M* Na_2CO_3 gave rise to an absorption band with λ_{max} 310 mμ. Their observation was made in an effort to explain the anomalous reactivity of **2** under the conditions of the Willstätter–Schudel alkaline NaOI test. According to our scheme, **5** would consume 2 equiv of NaOI by substitution of the methylene hydrogens.