Strong evidence in favor of structure I for zygadenine has now been obtained by interrelation of zygadenine with germine. Treatment of 7-ketogermine-3,16-diacetate[8] in methanol with 1,3-propanedithiol and anhydrous hydrogen chloride yields 7-ketogermine-3,16-diacetate-propylene thioketal hydrochloride (VII), m.p. 265–266° dec., $[\alpha]^{23}D$ − 5° (py.); $\lambda_{max.}^{alc.}$. 246 mμ (ε 600). Found: C, 56.86; H, 7.80; S, 9.13; Cl, 5.11. Raney nickel desulfurization of VII affords zygadenine-3,-16-diacetate (V), characterized by mixed melting point and infrared spectral comparison with the authentic sample.[9]

(9) This work was supported by a grant from the National Heart Institute of the National Institutes of Health (H-2275).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN S. MORRIS KUPCHAN
MADISON, WISCONSIN

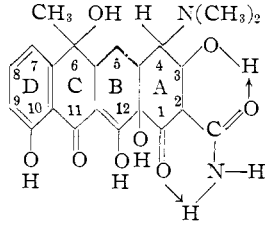
RECEIVED APRIL 20, 1956

ON THE NATURE OF THE REVERSIBLE ISOMERIZATIONS OCCURRING IN THE TETRACYCLINE FAMILY

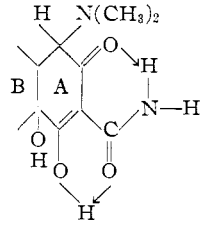
Sir:

We have recently reported that each of the known tetracyclines can be reversibly converted to a new, isomeric substance. These new substances were named the quatrimycins.[1] We now wish to report further studies on the nature of these reversible changes.

Consideration of the ultraviolet spectral differences between pair members[1,2] and of the conditions permitting and preventing isomerization[1] makes it most likely that the isomerization involves only a change in the configuration of C.4. However, changes in the orientation of the carboxamide group (I and II below), as suggested previously[3] to



I



II

explain the existence of α- and β-apoterramycin, are not entirely excluded. In an attempt to exclude the latter possibility, tetracycline[4] and quatrimycin were converted to the benzenesulfonyl nitriles[3] and chlorotetracycline[4] and chloroquatrimycin were converted to the unsubstituted nitriles.[5] Benzenesulfonyltetracyclinonitrile dimethylformamide (DMF) solvate: $[\alpha]^{25}D$ −416° (0.5% in DMF); m.p., dec. above 210°; *Anal.* Calcd. for $C_{31}H_{34}N_3SO_{10}$: C, 58.20; H, 5.31; N, 6.56; S, 5.00. Found: C, 58.25; H, 5.24; N, 5.93; S, 4.93. Benzenesulfonylquatrimycinonitrile monohydrate: $[\alpha]^{25}D$ −336° (0.5% in DMF); m.p., dec. above 200°; *Anal.* Calcd. for $C_{28}H_{29}N_2SO_{10}$: C, 57.40; H, 4.96; N, 4.78; S, 5.47. Found: C, 57.51; H, 5.29; N, 4.74; S, 4.97. Chlorotetracyclinonitrile: $[\alpha]^{25}D$ −338° (0.5% in DMF); m.p., dec. above 220°; *Anal.* Calcd. for $C_{22}H_{21}N_2O_7Cl$: C, 57.30; H, 4.56; N, 6.07; Cl, 7.69. Found: C, 56.99; H, 5.01; N, 6.37; Cl, 7.37. Chloroquatrimycinonitrile monohydrate: $[\alpha]^{25}D$ −300° (0.5% in DMF); m.p., dec. above 190°; *Anal.* Calcd. for $C_{22}H_{23}N_2O_8Cl$: C, 55.10; H, 4.80; N, 5.85; Cl, 7.40. Found: C, 54.91; H, 4.76; N, 5.72: Cl, 7.89. The members of each nitrile pair are isomeric and distinguishable[6] but none of these four compounds could be isomerized, although many conditions were tried. Thus, the carboxamide group, though not essential to the existence of isomeric pairs, does play a part in the ready interconversion of the parent compounds. Interconversion of the nitrile pair members under conditions equilibrating the tetracyclines, had it been possible, would have provided a simple, certain proof that the sulfonyl chloride reagents had acted in completely parallel ways on both members of the parent pairs. Lack of this proof weakens somewhat the argument that distinguishable sulfonyl chloride reaction products have eliminated the carboxamide orientation possibility.

Further work to establish epimerization at C.4 took the form of eliminating the asymmetry at C.4 by reductive removal of the 4-dimethylamino group. Zinc and glacial acetic acid at 30° for six hours can accomplish this.[2] However, we have found that both tetracycline and quatrimycin are completely equilibrated in 2.5 hours in glacial acetic acid–zinc acetate at 25°. Thus, even if reduction under these conditions yielded identical desdimethylamino products from both members of an isomeric pair, no distinction could be drawn between the alternatives of isomerization during reduction and of configuration at C.4 being the difference separating the pair members. This difficulty was resolved by preparing the methiodides of tetracycline and quatrimycin and reducing them in 50% aqueous acetic acid.[7] Tetracycline methiodide: $[\alpha]^{25}D$ −198° (0.5% in 0.03*N* HCl); m.p., 178–180° (dec.); *Anal.* Calcd. for $C_{23}H_{27}N_2O_8I$: C, 47.11; H, 4.64; N, 4.71; I, 21.64. Found: C, 47.38; H, 4.58; N, 4.91; I, 22.15. Quatrimycin methiodide: $[\alpha]^{25}D$ −265° (0.5% in 0.03 *N* HCl); m.p., 161–162° (dec.); *Anal.* Found: C, 47.32; H, 4.74; N, 4.64; I, 21.29. The two methiodides were isomeric, distinguishable, and reversibly interconvertible. However, neither methiodide was measurably isomerized after remaining four hours in a reduction solvent consisting of 50% aqueous acetic acid with four equivalents of zinc acetate;

(1) A. P. Doerschuk, B. A. Bitler and J. R. D. McCormick, THIS JOURNAL, **77**, 4687 (1955).

(2) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **76**, 3568 (1954).

(3) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

(4) The trademarks of the American Cyanamid Co. for tetracycline and chlorotetracycline are Achromycin and Aureomycin, respectively.

(5) This method, involving reaction with methanesulfonyl chloride and pyridine, is that of R. Wilkinson, Research Division, American Cyanamid Company, who had used it previously to prepare the nitrile of chlorotetracycline.

(6) Ultraviolet and infrared spectra and solubilities were used as criteria of distinguishability.

(7) The methods of methiodide preparation and reduction and a sample of tetracycline methiodide were obtained from J. Boothe, Research Division, American Cyanamid Co.

reduction in 50% aqueous acetic acid with zinc for forty-five minutes was sufficient for reductive removal of the trimethylammonium group from both methiodides. The two resulting desdimethylamino products were identical. Desdimethylaminotetracycline: $[\alpha]^{25}D$ −250° (0.5% in methyl cellosolve); m.p. 195° (dec.); *Anal.* Calcd. for $C_{20}H_{19}NO_8$: C, 59.75; H, 4.77; N, 3.49. Found: C, 59.80; H, 4.72; N, 3.30. "Desdimethylaminoquatrimycin": $[\alpha]^{25}D$ −251° (0.5% in methyl cellosolve); m.p., 195° (dec.); *Anal.* Found: C, 59.40; H, 4.88; N, 3.42. Mixed melting point showed no depression.[6]

This represents necessary and sufficient proof that the quatrimycins are the 4-*epi*tetracyclines.

J. R. D. McCORMICK
SIDNEY M. FOX
CHEMICAL PROCESS IMPROVEMENT DEPT. LELAND L. SMITH
LEDERLE LABORATORIES DIVISION BARBARA A. BITLER
AMERICAN CYANAMID COMPANY JULES REICHENTHAL
PEARL RIVER, NEW YORK VICTOR E. ORIGONI
WALTER H. MULLER
ROBERT WINTERBOTTOM
ALBERT P. DOERSCHUK

RECEIVED MARCH 5, 1956

THE BIOSYNTHESIS OF α, ε-DIAMINOPIMELIC ACID. I. ISOLATION OF AN INTERMEDIATE, ACTIVE FOR A DIAMINOPIMELIC ACID-REQUIRING *E. coli* MUTANT

Sir:

It has been well established[1] that α,ε-diaminopimelic (DAP) acid is found in many Gram-negative and some Gram-positive organisms. The biosynthetic mechanism of DAP synthesis is at present unknown and it is the purpose of this communication to report the isolation of a biologically active compound apparently formed as an intermediate in the biosynthesis of DAP.

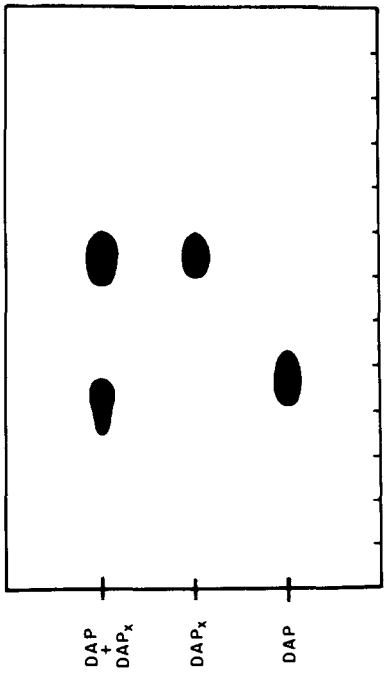
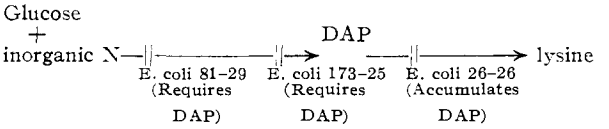


Fig. 1.—The chromatographic behavior of a material having growth-supporting activity for a DAP-requiring *E. coli* mutant: solvent, methanol (80), water (20), pyridine (4); temp. 25; descending system, Whatman No. 1 paper, bioautographic plate, using *E. coli* 81-29.

(1) E. Work and D. L. Dewey, *J. Gen. Microbiol.*, **9**, 394 (1953).

The biosynthesis of DAP can be accomplished by *E. coli*, using glucose as a sole source of carbon. Using the mutant[2] system illustrated below, it has been possible to isolate a preparation, active for a DAP-requiring mutant, *E. coli* 81–29.



This biologically active material was designated as DAP_x for convenience.

DAP_x is extracted from lyophilized *E. coli* 26–26 supernates at *p*H 3–5 with diethyl ether, methylene chloride and *n*-butanol. The solvents are removed *in vacuo* and the solids dissolved in water and freeze-dried to yield a yellowish-brown product. Chromatography of DAP_x in a methanol–water–pyridine system and subsequent analysis on a bioautographic plate (Fig. 1) demonstrated that the material was different from DAP.

The material was found to be ninhydrin-negative, heat stable and acidic in character. Countercurrent distribution using *n*-butanol and water at *p*H 3 yielded a highly active fraction which was obtained in a pure state by crystallization from *n*-butanol. The material was identified as succinic acid by its infrared spectrum and by comparison of the free acid (m.p. and mixed m.p. 187.5–188.5°) and its *p*-bromophenacyl ester (m.p. and mixed m.p. with 214–215°) authentic specimens.

Subsequent studies with *E. coli* 26–26 have shown that aspartic acid, succinic acid, pyruvic acid, triphosphopyridine nucleotide (TPN) and adenosine triphosphate (ATP) stimulate the synthesis of DAP by cell-free extracts. The stimulation of DAP synthesis by the compounds described above extend and confirm the report of Gilvarg[3] which appeared at the time this manuscript was in preparation.

Further studies on the biosynthetic mechanism of DAP synthesis are in progress and will be a subject of future publication.

(2) The three *E. coli* mutants were kindly obtained from Dr. B. Davis, New York University.

(3) C. Gilvarg, *Fed. Proc.*, 261 (1956).

RESEARCH LABORATORIES
THE UPJOHN COMPANY LIONEL E. RHULAND
KALAMAZOO, MICH. BRIAN BANNISTER

RECEIVED MAY 21, 1956

A NEW TWO STRANDED HELICAL STRUCTURE: POLYADENYLIC ACID AND POLYURIDYLIC ACID

Sir:

While studying the X-ray diffraction patterns of synthetic nucleotide polymers, we mixed together the sodium salts of polyadenylic acid and polyuridylic acid.[1] There resulted a very rapid

(1) M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).