spectrum of structural changes with relatively mild reagents; the reactions are summarized in Fig. 1.[4]

In warm glacial acetic acid solution the bridging reaction is reversed and the diazepinone II is formed. This acyl transfer is reminiscent of the deacetylation of imido esters which occurs under these conditions.[5] In aqueous methanolic hydrochloric acid, the bicyclic system rearranges *without hydrolysis* to furnish the 1-benzamidopyridinium chloride V[6] (Found: C, 67.21; H, 5.16; N, 8.30) in 90% yield. This reaction can be depicted very simply as an allylic shift of the bridging bond from C–5 to C–3, initiated by protonation at N–1, followed by collapse of the resulting 1,7-diazabicyclo[4.1.0]heptanone IV to V.

On brief refluxing in methanol solution containing a catalytic amount of benzoic or *p*-toluenesulfonic acid, a product having the composition of a hemiketal is obtained (Found: C, 71.30; H, 5.99; N, 8.31; OCH_3, 9.23). This compound is similarly convertible to II and V, but from ultraviolet (λ_{max}^{EtOH} 261 mμ) and other data it appears not to be the bicyclo[3.2.0]hemiketal derived from III by simple carbonyl addition. (Carbonyl derivatives of III, such as the semicarbazone, have not been obtained despite numerous attempts).

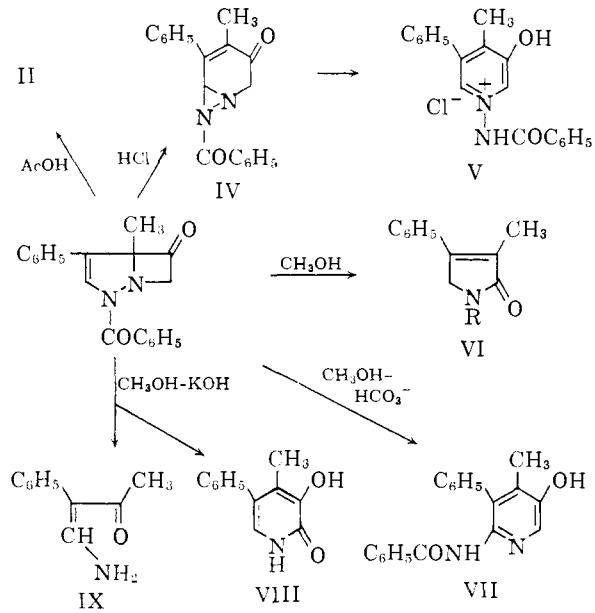
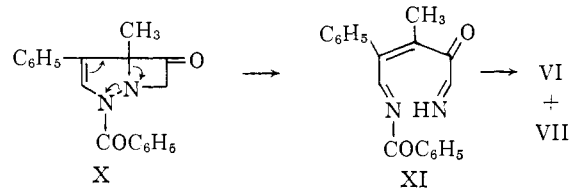
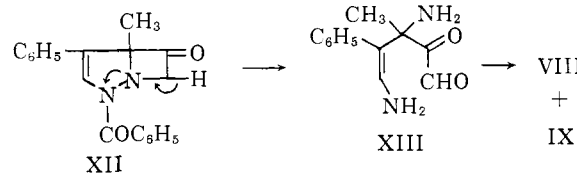


Fig. 1

Refluxing III in neutral methanol solution (containing benzoic acid and sodium benzoate) gives the 1-benzoylpyrrolone VI (R = C_6H_5CO) (Found: C, 78.39; H, 5.70; N, 5.31) in 40% yield. This substance was characterized by mild alkaline hydrolysis to the pyrrolone VI (R = H).[3] In methanol solution containing a catalytic amount of ammonia or bicarbonate, the major product (70%) is the isomeric 6-benzamidopyridine VII.[7] In stock methanol, without added reagents, both VI and VII are obtained, and it appears that these compounds may arise from a common precursor. One possibility is the concerted rupture of the N–N and C–5—N bonds, as in X → XI, then recyclization by two different paths, although the driving force for these reactions is not obvious. A polar solvent is necessary; heating III in benzene or toluene leads to another, highly reactive isomer whose structure is not yet known.



In cold methanolic potassium hydroxide a mixture of products is formed from III which includes the enamine IX,[8] m.p. 98° (Found: C, 74.42; H, 7.13; N, 8.60) (35% yield), characterized by conversion to 3-methyl-4-phenylpyrazole and by synthesis from 1-phenyl-1-ethoxymethyleneacetone, an unidentified compound $C_{17}H_{17}O_2N$ (6%), and a compound (5%) which has been identified as the pyridone VIII (Found: C, 71.13; H, 5.58; N, 7.44) by comparison with a product obtained by Elbs oxidation of 3-hydroxy-4-methyl-5-phenylpyridine[9] (the other possible oxidation product, the previously known[7] 3-hydroxy-4-methyl-5-phenyl-6-pyridone, also was obtained). The cleavage observed in this reaction probably is initiated by removal of a proton at C–7 followed by elimination at the N—N bond (XII) and hydrolysis to give a species such as XIII which can be conceived as a common precursor of VIII and IX. Dismutation of the α-dicarbonyl system and ring closure would furnish VIII; fragmentation with loss of glyoxal and further hydrolysis would give IX.



Further discussion and details of these reactions will be presented in forthcoming papers.

(4) Most of the reactions described have also been observed with the 2-acetyl derivative as well.

(5) W. Z. Heldt, *J. Am. Chem. Soc.*, **80**, 5880 (1958).

(6) J. A. Moore and J. Binkert, *ibid.*, **81**, 6045 (1959).

(7) J. A. Moore and F. J. Marascia, *ibid.*, **81**, 6050 (1959).

(8) Lit. m.p. 96°, H. Rupe, A. Metzger and H. Vogler, *Helv. Chim. Acta*, **8**, 848 (1925).

(9) J. A. Moore and H. Puschner, *J. Am. Chem. Soc.*, **81**, 6041 (1959).

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BIOSYNTHESIS OF THE TETRACYCLINES. IV.[1] BIOLOGICAL REHYDRATION OF THE 5a,6-ANHYDROTETRACYCLINES

Sir:

In our studies on the pathways of biosynthesis of the tetracyclines, we have examined, for pre-

(1) Previous series paper: J. R. D. McCormick, J. Reichenthal, U. Hirsch and N. O. Sjolander, *J. Am. Chem. Soc.*, **84**, in press (1962).

TABLE I

BIOLOGICAL TRANSFORMATIONS OF 5a,6-ANHYDROTETRACYCLINE DERIVATIVES

Substrate	Streptomyces strain used	Product observed
C^{14}-5a,6-Anhydrotetracycline[a]	*S. aureofaciens* BC-41[b]	C^{14}-Tetracycline[a,c]
C^{14}-5a,6-Anhydrotetracycline	*S. rimosus* T1686B[d]	C^{14}-5-Hydroxytetracycline[e]
C^{14}-5a,6-Anhydro-7-chlorotetracycline[f]	*S. rimosus* T1686B	Starting material only
Cl^{36}-5a,6-Anhydro-7-chlorotetracycline	*S. aureofaciens* BC-41	Cl^{36}-7-Chlorotetracycline[f]
Cl^{36}-5a,6-Anhydro-7-chlorotetracycline	*S. aureofaciens* S1308[g]	Cl^{36}-7-Chloro-5a,11a-dehydrotetracycline[g]
5a,6-Anhydro-6-demethyltetracycline[h]	*S. aureofaciens* S2242[i]	6-Demethyltetracycline[j]
C^{14}-5a,6-Anhydro-6-demethyltetracycline	*S. rimosus* T1686B	Starting material only
5a,6-Anhydro-7-chloro-6-demethyltetracycline[k]	*S. aureofaciens* S2311[l]	7-Chloro-6-demethyltetracycline[j]
5a,6-Anhydro-5-hydroxytetracycline[e]	*S. aureofaciens* V828[m]	5-Hydroxytetracycline[e]
5a,6-Anhydrotetracyclinonitrile[n]	*S. aureofaciens* T219[o]	Tetracyclinonitrile[n]
5a,6-Anhydro-2-acetyl-2-decarboxamidotetracycline[p]	*S. aureofaciens* S2242	2-Acetyl-2-decarboxamidotetracycline[q]
5a,6-Anhydro-4-*epi*tetracycline[r]	*S. aureofaciens* T-219	Starting material only
5a,6-Anhydro-7-chloro-4-*epi*tetracycline[r]	*S. aureofaciens* T219	Starting material only
5a,6-Anhydro-4-dedimethylaminotetracycline[s]	*S. aureofaciens* T219	Starting material only
5a,6-Anhydro-12a-deoxytetracycline[t]	*S. aureofaciens* T219	12a-Deoxytetracycline[t]

[a] 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, *J. Am. Chem. Soc.*, **76**, 3568 (1954). [b] A 7-chlorotetracycline-producing strain: A. P. Doerschuk, J. R. D. McCormick, J. J. Goodman, S. A. Szumski, J. A. Growich, P. A. Miller, B. A. Bitler, E. R. Jensen, M. Matrishin, M. A. Petty and A. S. Phelps, *ibid.*, **81**, 3069 (1959). [c] 7-Chlorotetracycline was also produced but was not labeled. [d] A 5-hydroxytetracycline-producing soil isolate obtained from the Lederle Laboratories Biochemical Research Section. [e] See reference 4. [f] See reference 7. [g] A 7-chloro-5a,11a-dehydrotetracycline-producing strain: J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander and A. P. Doerschuk, *J. Am. Chem. Soc.*, **80**, 5572 (1958). [h] 5a,6-Anhydro-6-demethyltetracycline was prepared by the method used by Webb and co-workers (see footnote *k*) in the preparation of 5a,6-anhydro-7-chloro-6-demethyltetracycline. [i] A non-producing mutant derived from a 7-chlorotetracycline-producing strain: J. R. D. McCormick, U. Hirsch, N. O. Sjolander and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 5006 (1960). [j] J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, *ibid.*, **79**, 4561 (1957). [k] J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein and C. F. Wolf, *ibid.*, **79**, 4564 (1957). [l] A tetracycline-producing, non-7-chlorotetracycline-producing strain (supplied by Dr. J. Growich). [m] A non-producing strain (supplied by Miss U. Hirsch) descended from a 7-chlortetracycline-producing strain. [n] Prepared by the method of R. Wilkinson, see reference 15 in footnote *r*. [o] A non-producing strain derived from a tetracycline-producing strain: see reference in footnote *i*. [p] Prepared from 2-acetyl-2-decarboxamidotetracycline (see footnote *q*). *anal.*, found for $C_{23}H_{23}NO_7 \cdot HCl \cdot {}^1/_2H_2O$: C, 58.4; H, 5.44; N, 3.37; Cl, 7.45. [q] J. R. D. McCormick and co-workers, unpublished work; and M. W. Miller and F. A. Hochstein, presented before the division of Medicinal Chemistry at the Chicago meeting of the American Chemical Society, Sept., 1961. [r] J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 2849 (1957). See reference 5. [s] See reference 8.

cursor activity, several of the more readily available chemical degradation products of the tetracyclines. Two methods of examination have been used: (1) appearance of a labeled tetracycline in a fermentation system to which a labeled degradation product had been added or (2) appearance, in the fermented mash, of a tetracycline derivative related to the substance added and different from the normal products of the particular strain used. A selected group of tetracyclines, producing and non-producing strains of *Streptomyces aureofaciens* and *Streptomyces rimosus*, was used (Table I).

The degradation products were added to shaker flask fermentations at 24 to 48 hours after inoculation, as dry solids in concentrations averaging about 400 mcg./ml. After an additional 72 to 96 hours of incubation, the products, or recovered starting materials, were detected in the fermented mashes by specific chemical assays, by antibacterial assay, by paper chromatography, and/or by radiochemical means.

Of the compounds tested, only 5a,6-anhydrotetracyclines showed significant precursor activity; conversion yields of rehydrated product were as high as 75%. As shown in Table I, however, not all such compounds were active. Of particular significance are the failures of 5a,6-anhydrodedimethylaminotetracycline and 5a,6-anhydro-4-*epi*-tetracycline, indicating that the presence of the dimethylamino group in the proper configuration is essential for the rehydration observed, and that the process is a biological one involving specific attachment to enzyme receptor sites.

Also significant is the observation that, in one case, the rehydration was accompanied by, or probably preceded by, 5-hydroxylation[2]: 5a,6-anhydrotetracycline was converted by *S. rimosus* to 5-hydroxytetracycline. Here again the transformation appeared to have severe structural requirements in that attempts to produce 7-chloro-5-hydroxytetracycline from 5a,6-anhydro-7-chlorotetracycline and 6-demethyl-5-hydroxytetracycline from 5a,6-anhydro-6-demethyltetracycline with *S. rimosus* were not successful; and indeed, these latter anhydro compounds were left unaffected, being neither hydroxylated nor rehydrated by *S. rimosus*.[3]

No other simple transformations were observed to accompany the rehydration. It can be seen from the results in Table I that no 7-chlorination,

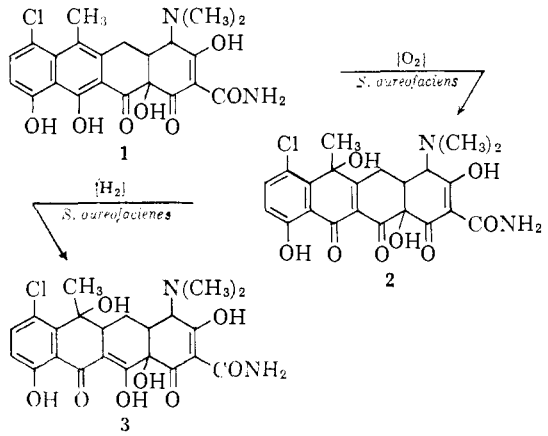
(2) We have found that C^{14}-tetracycline was not hydroxylated to 5-hydroxytetracycline under the conditions used here (unpublished work).

(3) The conversion of 5a,6-anhydrotetracycline to 5-hydroxytetracycline by *S. rimosus*, the indifference of this organism toward some other 5a,6-anhydrotetracyclines, and the conversion by *S. aureofaciens* of 5a,6-anhydro-5-hydroxytetracycline to 5-hydroxytetracycline could all be accounted for by the assumptions (a) that 5-hydroxylation precedes the 5a,6-hydration; (b) that the structural requirements for 5-hydroxylation are highly specific; and (c) that the rehydration system of *S. rimosus* requires the presence of a 5-hydroxyl group.

6-methylation, 12a-hydroxylation, 2-acetyl to 2-carboxamide conversion, nor 2-nitrile hydrolysis ($CN \rightarrow CONH_2$) was observed. In addition, no biological transformation products were detected from the following compounds: apoterramycin[4]; dedimethylaminotetracycline[5]; 4a,12a-anhydrotetracycline[6]; isoaureomycin[7]; dedimethylamino-12a-deoxytetracycline[5]; and 12a-deoxytetracycline.[8]

The observed rehydration of 5a,6-anhydro-12a-deoxytetracycline to 12a-deoxytetracycline, unaccompanied by 12a-hydroxylation, demonstrates that neither of these compounds is an intermediate in the biosynthetic pathway and that the presence of 12a-hydroxyl is unimportant in the rehydration reaction.[9]

The rehydration of the 5a,6-anhydrotetracyclines is probably the net result of a biological oxidation and then a biological reduction. Thus the apparent rehydration of 5a,6-anhydro-7-chlorotetracycline (**1**) to 7-chlorotetracycline (**3**) would be the result first, of oxidative hydroxylation to yield 7-chloro-5a,11a-dehydrotetracycline (**2**) followed by biological reduction[10] of the 5a,11a double bond yielding **3**. This was confirmed by the conversion



of Cl^{36}-5a,6-anhydro-7-chlorotetracycline to Cl^{36}-7-chloro-5a,11a-dehydrotetracycline by *S. aureofaciens* S1308, a mutant blocked for the reduction of **2** to **3**. We therefore conclude that oxidative hydroxylation of 5a,6-anhydrotetracyclines at C–6 and reduction of the resulting 5a,11a-dehydrotetracyclines are the final two steps in the biosynthetic pathways to the tetracyclines.[11,12]

(4) 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, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

(5) A. Green, R. G. Wilkinson and J. H. Boothe, *ibid.*, **82**, 3946 (1960).

(6) K. Blackwood, H. H. Rennhard and C. R. Stephens, *ibid.*, **82**, 745 (1960).

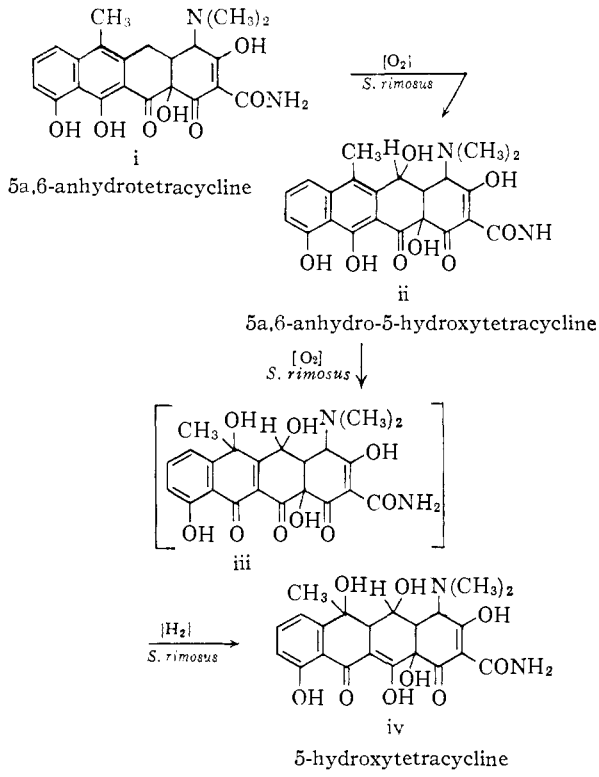
(7) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.*, **74**, 4981 (1952).

(8) A. Green and J. H. Boothe, *ibid.*, **82**, 3950 (1960).

(9) Although C. E. Holmlund, W. W. Andres and A. J. Shay [(*ibid.*, **81**, 4750 (1959)] were able to demonstrate the 12a-hydroxylation of 12a-deoxytetracycline by *Curvularia lunata*, they too were unable to demonstrate a similar 12a-hydroxylation by *S. aureofaciens*.

(10) J. R. D. McCormick, N. O. Sjolander, P. A. Miller, U. Hirsch, N. H. Arnold and A. P. Doerschuk, *ibid.*, **80**, 6460 (1958).

(11) Similarly, the last steps in the biosynthesis of 5-hydroxytetracycline would appear as:



although at this time experimental evidence for the intermediate iii is lacking.

(12) NOTE ADDED IN PROOF.—A. I. Scott and C. T. Bedford [*J. Am. Chem. Soc.*, **84**, 2271 (1962)] have now reported the photooxidation of 5a,6-anhydro-7-chlorotetracycline to the 6-hydroperoxide and the reduction of this product to 7-chloro-5a,11a-dehydrotetracycline in simulation of the biosynthetic process.

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RECEIVED JUNE 7, 1962

A REINVESTIGATION OF THE APPLICABILITY OF THE SELECTIVITY RELATIONSHIP TO SE REACTIONS OF BIPHENYL AND FLUORENE

Sir:

The non-adherence of the rates of electrophilic aromatic substitution reactions in the *para* position of biphenyl to the Selectivity Relationship recently has been clearly demonstrated.[1] The anomalously small activation by the phenyl group, and the increase in its activating ability with increase in electron demand of the attacking electrophile have been rationalized on the basis of the variable noncoplanar configuration of the two benzene rings.[1] This theory is supposedly further substantiated by the much greater reactivity of fluorene and the adherence of the latter compound to the Selectivity Relationship in SE reactions. In fact, a reinvestigation of the available data for SE reactions of fluorene[1] shows an apparent *deviation* from the Selectivity Relationship *analogous to that of biphenyl* (see Fig. 1).

(1) L. M. Stock and H. C. Brown, *J. Am. Chem. Soc.*, **84**, 1242 (1962), and H. C. Brown, *et al.*, *ibid.*, **84**, 1229, 1233, 1236 and 1238 (1962).