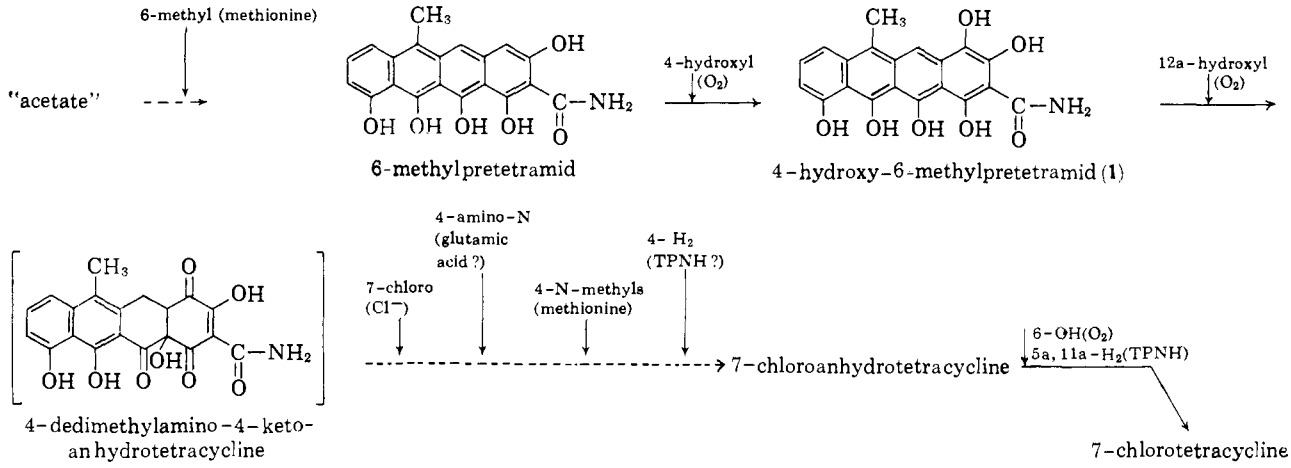
Chart I



amide, or, in accordance with the nomenclature which we have suggested for this family of compounds,[5] 4-hydroxy-6-methylpretetramid (**1**). This compound was isolated from V655 freeze-dried, whole mash by extraction into tetrahydrofuran and was crystallized from that solvent by evaporation. It was obtained as orange needles of no definite melting point. Analyses indicated the composition $C_{20}H_{15}NO_7$. When the compound was dissolved in dimethyl sulfoxide and added to a 24-hr. ED1369 fermentation, up to 75% of the theoretical amount of 7-chlorotetracycline was formed on further incubation.

The fermentation conditions for the accumulation of **1** were generally those found best for other *S. aureofaciens* fermentations,[6] and the amount accumulated was about 500 μg./ml.

The discovery of this mutant-produced, naphthacenic intermediate is full confirmation of the postulated role of completely aromatic naphthacene derivatives as intermediates in the biosynthesis of the tetracyclines.[5] The details of structure of **1**, *i.e.*, the presence of the 4-hydroxyl and the 6-methyl groups and the absence of the 7-chloro substituent, confirm our earlier conclusions about the relative locations of the introduction of the 6-methyl and 7-chloro substituents[5] and strongly suggest the manner of introduction of the dimethylamino group. Our current assessment of this portion of the pathway from "acetate" to 7-chlorotetracycline is shown in Chart I.

(5) J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1692 (1963).

(6) (a) J. R. D. McCormick, J. Reichenthal, U. Hirsch, and N. O. Sjolander, *ibid.*, **84**, 3711 (1962); (b) J. J. Goodman, U. S. Patent 3,050,446 (1962).

(7) The partial biosynthetic scheme presented here encompasses conclusions drawn from the following works as well as from the present paper: R. Robinson, "Structural Relations of Natural Products," Oxford Press, 1955; S. Gatenbeck, *Biochem. Biophys. Res. Commun.*, **6**, 422 (1961); J. F. Snell, A. J. Birch, and P. L. Thomson, *J. Am. Chem. Soc.*, **82**, 2402 (1960); ref. 5, this paper; 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, *J. Am. Chem. Soc.*, **81**, 3069 (1959); P. A. Miller, A. Saturnelli, J. H. Martin, L. A. Mitscher, and N. Bohonos, *Biochem. Biophys. Res. Commun.*, **16**, 285 (1964); J. R. D. McCormick, P. A. Miller, S. Johnson, N. Arnold, and N. O. Sjolander, *J. Am. Chem. Soc.*, **84**, 3023 (1962).

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Biosynthesis of the Tetracyclines. VIII.[1] Characterization of 4-Hydroxy-6-methylpretetramid[2]

Sir:

As presented in the previous communication[1] 4-hydroxy-6-methylpretetramid (**1**), a substance biologically convertible to 7-chlorotetracycline, was isolated from the mash of a *Streptomyces aureofaciens* mutant, V655. The compound was separated by extraction with tetrahydrofuran and was crystallized from this solvent by evaporation. Product of high purity was obtained as orange needles by recrystallization from hot phenol in the presence of hydriodic and hypophosphorous acids to prevent oxidation. The pure substance melted with decomposition over the range 260–310°. Analyses indicated the composition to be $C_{20}H_{15}NO_7$ (found: C, 63.1; H, 4.13; N, 3.78). The infrared absorption spectrum was strongly reminiscent of that of 6-methylpretetramid (desdimethylaminoterrarubein[3]) but was distinguished by the presence of a strong sharp maximum at 970 cm.$^{-1}$. The absorption spectrum in sulfuric–boric acid[4] was very characteristic [λ_{max} (ϵ): 280 (22,100), 316 (42,100), 467 (15,400), and 520 mμ (16,500)], and again was similar to, but easily distinguished from, that of 6-methylpretetramid under the same conditions [λ_{max} (ϵ): 263 (22,900), 278 (21,600), 341 (14,200), 400 (14,700), and 512 mμ (13,700)].

Like the earlier known pretetramid derivatives, crystalline **1** was essentially insoluble in most organic solvents but dissolved readily in dimethyl sulfoxide containing 1% magnesium acetate tetrahydrate, forming an amber solution. The resulting solution was stable in the absence of oxygen, but in the presence of air was oxidized. The uptake of oxygen was initially rapid, but slowed and finally came to halt after about 6 hr. at 25°. The final solution was purple, and the absorp-

(1) Paper VII: J. R. D. McCormick, U. H. Joachim, E. R. Jensen, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **87**, 1793, (1965).

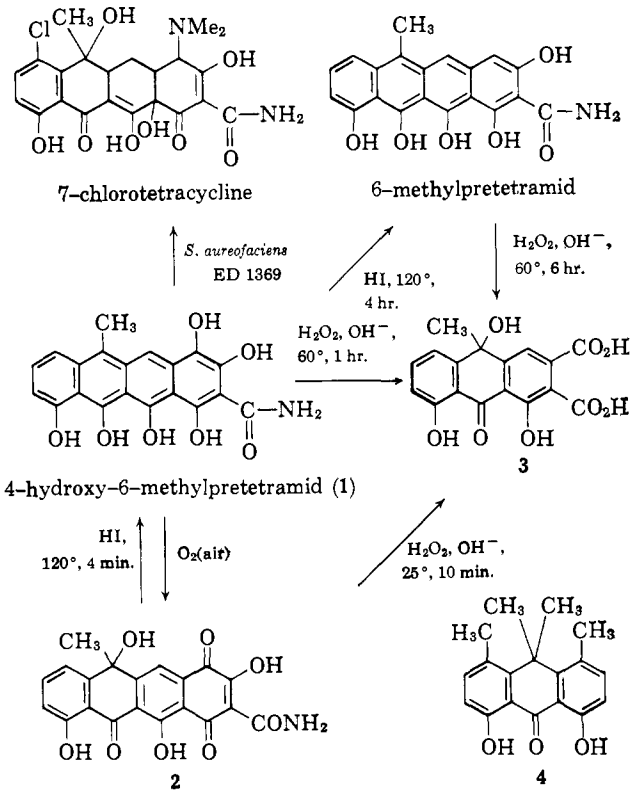
(2) A preliminary report of the material of this communication formed part of a paper presented at the Congress for Antibiotics, Prague, Czechoslovakia, June 1964.

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

(4) The solvent was 99 parts concentrated (97%) sulfuric acid and 1 part saturated aqueous sodium tetraborate. Spectrophotometric solutions were allowed to stand 0.5 hr. at room temperature before spectra were run.

tion spectrum of the solution at this point showed λ_{max} (ϵ) 283 (6260) and 540 mμ (10,400). Dilution of the solution with 0.1 *N* aqueous hydrochloric acid yielded crystalline 1,4,6,11-tetrahydro-3,6,10,12-tetrahydroxy-6-methyl-1,4,11-trioxonaphthacene-2-carboxamide (**2**). *Anal.* Found for $C_{20}H_{13}NO_8$: C, 60.9; H, 3.44; N, 3.29; C-methyl, 2.6. The dark red, very finely crystalline product did not melt, but decomposed over the range 200–300°. The structure of **2** was elucidated by its ready reduction back to **1** and by the easy further oxidation with alkaline hydrogen peroxide to racemic 1,8,10-trihydroxy-10-methylanthrone-2,3-dicarboxylic acid (**3**); yellow needles (acetone solvate) from acetone–benzene; m.p. 140° dec. ($-H_2O$). *Anal.* Found for $C_{17}H_{12}O_8 \cdot 0.5C_3H_6O$: C, 59.6; H, 4.43; acetone, 0.47 mole (as iodoform). This product, **3**, was also prepared by the slower oxidation in alkaline hydrogen peroxide of 6-methylpretetramid itself. Spectra of specimens of **3** from each starting material were identical, λ_{max} (ϵ) (0.1 *N* hydrochloric acid in methanol): 255 (shoulder, 6340), 265 (7500), 274 (7680), 304 (9300), and 376 mμ (9300); infrared maximum (KBr disk): 1710 cm.$^{-1}$ (carboxyl C=O) and 1600 cm.$^{-1}$ (hydrogen bonded aryl C=O). The structure of **3** was confirmed by the detailed similarity of its ultraviolet absorption spectrum to that of 1,8-dihydroxy-4,5,10,10-tetramethylanthrone (**4**) [λ_{max} (ϵ) (0.1 *N* hydrochloric acid–methanol): 252 (shoulder, 6350), 260 (8890), 268 (9340), 300 (13,500), and 371 mμ (10,100)], which had been prepared earlier in these laboratories as a model compound for spectral comparisons.[5]

Scheme I. Reactions of 4-Hydroxy-6-methylpretetramid



(5) J. R. D. McCormick and W. E. Gardner, unpublished work. 1,8-Dihydroxyanthrone was allowed to react with methyl iodide in aqueous alkaline solution to yield a complex mixture of C-methylated products from which the cryptophenolic 4,5,10,10-tetramethyl derivative was isolated by exhaustive recrystallization from methanol and acetic acid, giving yellow platelets, m.p. 173–175°; infrared maximum at 1600 cm.$^{-1}$ (hydrogen bonded aryl C=O); n.m.r. (60 Mc., in $CDCl_3$ *vs.* TMS): six-proton singlet at 98 c.p.s. (alkyl methyls), six-proton singlet at 136 c.p.s. (aryl methyls), four protons in a characteristic pair of doublets centered at 421 and 444 c.p.s. (vicinal pairs of aryl protons), and a two-proton singlet at 780 c.p.s. (phenolic OH). *Anal.* Found for $C_{18}H_{18}O_8$: C, 77.1; H, 6.6; C-methyl, 13.2; O-methyl, 0.00.

Upon mild reduction (hydriodic acid in phenol, 120°, 4 min.), the quinone **2** was reduced back to **1**, which in turn, under more vigorous conditions (HI, phenol, 120°, 4 hr.), was further reduced to 6-methylpretetramid. (We have previously observed that terrarubein, 4-dimethylamino-6-methylpretetramid, is similarly reductively cleaved to 6-methylpretetramid under these vigorous conditions.[6])

The analyses, spectral relations, and transformations summarized in Scheme I identify **I** as 1,3,4,10,11,12-hexahydroxy-6-methylnaphthacene-2-carboxamide, that is, 4-hydroxy-6-methylpretetramid.[7]

(6) J. R. D. McCormick and J. Reichenthal, unpublished work.

(7) This compound has now been prepared by degradation of tetracycline: J. Hlavka, P. Bitha, and J. Boothe, *J. Am. Chem. Soc.*, **87**, 1795 (1965).

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4-Hydroxy-6-methylpretetramid.[1] Synthesis *via* Quaternary Tetracyclines

Sir:

The isolation and identification of 4-hydroxy-6-methylpretetramid (**5**), a precursor in the biosynthesis of tetracycline, has recently been described.[2] We wish to report on three synthetic approaches to this important biosynthetic intermediate. We have found that refluxing tetracycline methyl betaine[3] (**1**) in acetonitrile yields a new dedimethylamino derivative to which we have assigned the structure **2**, 4a,12a-anhydro-4-dedimethylamino-4-hydroxytetracycline; $\lambda_{max}^{0.1NHCl}$ 370 and 485 mμ (log ϵ 3.75 and 4.17); n.m.r.[4] showed nine nonexchangeable protons. *Anal.* Found for $C_{20}H_{17}NO_8$: C, 59.9; H, 4.6; N, 3.5.

On the other hand, when the betaine **1** was refluxed in methanol we obtained as the major products both the γ-lactone **3** and the ϵ-lactone **4**. The structure of **3** was based on composition and spectral properties; $\lambda_{max}^{0.1NHCl}$ 260 and 335 mμ (log ϵ 4.14 and 3.79); λ_{max}^{KBr} 5.61 μ. *Anal.* Found for $C_{20}H_{17}NO_8$: C, 60.4; H, 5.0; N, 3.5. The n.m.r. spectrum of this material **3** exhibited a singlet at τ 3.8 due to the lone proton[5] on

(1) The name "pretetramid" has been suggested for 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide: J. R. McCormick, S. Johnson, and N. Sjolander, *J. Am. Chem. Soc.*, **85**, 1694 (1963).

(2) J. R. McCormick, Congress on Antibiotics, Prague, Czechoslovakia, June 1964; *J. Am. Chem. Soc.*, **87**, 1793 (1965).

(3) J. Boothe, G. Bonvicino, C. Waller, J. Petisi, R. Wilkinson, and R. Broschard, *ibid.*, **80**, 1654 (1958).

(4) All n.m.r. spectra were measured in deuterated dimethyl sulfoxide with tetramethylsilane as the internal standard using a Varian Model A60 spectrometer.