trile, lit.[2] m.p. 89–90.5°, 2,4-dinitrophenylhydrazone, m.p. 183–184°, lit.[2] m.p. 181–182°.

Infrared absorption bands, in cm.$^{-1}$, for diketone I: 2920 (s), 2841 (m), 1742 (s), 1701 (w), 1445 (m), 1357 (w), 1305 (m), 1176 (s), 1136 (w), 1033 (w), 984 (m), 915 (w), 864 (w); for monoketone III: 2920–2850 (s), 1767 (s), 1724 (w), 1445 (s), 1430 (w), 1346 (w), 1220 (w), 1155 (w), 1124 (m), 1031 (w), 995–980 (m), 926 (m), 860 (w), 840 (w).

The diketone I gave only a monosemicarbazone, isolated in 94% yield as tiny white needles, m.p. 210–212°, from 50% ethanol. Its infrared spectrum was consistent with its assigned structure.

Anal. Calcd. for $C_{15}H_{23}N_3O_2$: C, 64.9; H, 8.3; N, 15.1. Found: C, 65.2; H, 8.6; N, 15.1.

7,7-Bis(methylmercapto)dispiro[5.1.5.1]tetradecane (IV).[2]—A Pyrex bomb containing freshly fused zinc chloride (13.4 g., 0.098 mole), ketone III (24 g., 0.116 mole), anhydrous sodium sulfate (13.4 g., 0.094 mole), and methyl mercaptan (123.2 g., 2.56 moles) was allowed to stand at room temperature for 48 hr. with occasional shaking. The excess mercaptan was allowed to boil off and the residue was extracted with ice–water and ether. The ether extract was washed with cold 5% aqueous sodium hydroxide solution, then with water. After drying over sodium sulfate, the ether was removed under reduced pressure. Crude solid, m.p. 111–114°, was recrystallized from acetonitrile to give 25.1 g. (76%) of IV, m.p. 117–119°.

Walborsky and Buchman[2] reported a 48% yield for this compound and a melting point of 83.5–84.5°. They did not report a sulfur analysis for their product.

Anal. Calcd. for $C_{16}H_{28}S_2$: C, 67.6; H, 9.9; S, 22.5. Found: C, 67.4; H, 9.9; S, 22.4.

Dispiro[5.1.5.1]tetradecane (V).[2]—The following were combined and refluxed for 48 hr.: dithioketal IV (24 g., 0.084 mole), no. 28 Raney nickel repeatedly washed with ethanol prior to use (720 g.), and 95% ethanol (2000 ml.). The Raney nickel was filtered off. The ethanolic filtrate was concentrated to about half its initial volume and diluted with water. The resultant milky solution was extracted thoroughly with petroleum ether (b.p. 60–90°). The extract was dried over sodium sulfate and solvent removed by distillation. A pale yellow liquid residue resulted, which was distilled under reduced pressure. The product began to distil at 154° (51 mm.), but, since it started to solidify in the condenser, the distillation was halted. The pot residue, which solidified on cooling, and the solid from the condenser weighed 14.0 g. (86% yield of V). After recrystallization from acetonitrile the product melted from 56.5–58°.

Walborsky and Buchman[2] reported a 17% yield of their hydrocarbon and different physical properties. It may be significant that their product was isolated by distillation from sodium, while the use of sodium was avoided during the isolation of this product.

Anal. Calcd. for $C_{14}H_{24}$: C, 87.4; H, 12.6; mol. wt., 192; R_M (molar refractivity), 60.76. Found: C, 87.4, 87.6; H, 12.7, 12.6; mol. wt., 190, 191 (Rast method); R_M, 59.50.

Infrared absorption bands, in cm.$^{-1}$: 2920–2850 (s), 1447 (s), 1430 (m), 1340 (w), 1285 (m), 1245 (w), 1145 (w), 1067 (w), 948 (m), 939 (m), 848 (m).

3-Cyclohexylspiro[cyclohexane-1,4-(2′-pyrazolin-5′-one)] (VI).—The procedure of Barton, *et al.*,[5] was followed, using 10 g. of sodium (0.40 g.-atom), 500 ml. of diethylene glycol, 50 g. of anhydrous hydrazine (1.56 moles), and 30.8 g. of diketone I (0.14 mole). A total of 25.2 g. (77%) of VI, glistening white plates having a bluish luster, m.p. 174–175°, was isolated from ethanol.

Anal. Calcd. for $C_{14}H_{22}N_2O$: C, 71.8; H, 9.5; N, 12.0. Found: C, 72.1; H, 9.5; N, 12.0.

The infrared spectrum (KBr pellet) exhibited N—H stretch at 3185 cm.$^{-1}$ (strong intensity) and at 3067 cm.$^{-1}$ (medium intensity), carbonyl stretch at 1695 cm.$^{-1}$, and broad absorption between 741 and 800 cm.$^{-1}$ attributed to secondary amide N—H deformation.[17]

To rule out the possibility that VI contained a primary amide group, it was subjected to N-methylation according to the procedure of Loudon and Ogg[24] applicable to cyclic amides. The crude reaction product, a yellow oil, showed about 15 area % of unchanged VI by v.p.c. A portion of the major reaction product was trapped out from the column and its infrared spectrum obtained. There was no absorption in the N—H stretch region, nor in the amide N—H deformation region, supporting the structure assignment of VI. The carbonyl absorption shifted very slightly toward higher frequency (1698 cm.$^{-1}$).

Dispiro[5.1.5.1]tetradecane-7,14-diol.—The procedure used by Walborsky[4] to prepare dispiro[4.1.4.1]dodecane-6,12-diol was adapted for the preparation, using a solution of lithium aluminum hydride (4.7 g., 0.125 mole) in 700 ml. of anhydrous ether, and 55 g. of diketone I (0.249 mole). After recrystallization from benzene–ligroin, 37 g. (66%) of VII was obtained, m.p. 174–175°.

Anal. Calcd. for $C_{14}H_{24}O_2$: C, 74.9; H, 10.8. Found: C, 75.0; H, 10.9.

cis–trans isomers are possible for the diol but no effort was made to determine whether it was the *cis* or *trans* glycol (*cf.* Walborsky[4]). However, after initial acidification of the hydrolysate, the aqueous phase had remained slightly turbid. It was made strongly acid with 6 *N* sulfuric acid, and again extracted with ether. This latter extract was worked up separately and provided 0.2 g. of a solid melting at 198–200°, presumably a higher-melting isomer.

Ditosylate of Dispiro[5.1.5.1]tetradecane-7,14-diol.—The procedure of Marvel and Sekera[25] was modified and adapted for this preparation. From 20.3 g. of diol was obtained 47.7 g. of crude ditosylate (darkens at 135°, m.p. 210–220°). Although this yield was nearly quantitative, attempts to recrystallize led to considerable loss of product. From tetrahydrofuran two crops were obtained: 8.7 g. of small, white, felted needles, m.p. 224–225° (clear brown melt); and a second crop, 6.5 g. of small lustrous plates, m.p. 238–240° (clear melt, not darkening until several degrees above the melting point). The high- and low-melting forms of the ditosylate can be accounted for on the basis of *cis–trans* isomerism. No effort was made to identify the specific stereoisomers. The yield of isolated, purified product was 15.2 g. (31%).

Anal. Calcd. for $C_{28}H_{36}O_6S_2$: C, 63.1; H, 6.8; S, 12.0. Found for low-melting form: C, 63.5; H, 6.8; S, 12.3. Found for high-melting form: C, 63.2; H, 6.8; S, 12.0.

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(24) J. D. Loudon and J. Ogg, *J. Chem. Soc.*, 739 (1955).

(25) C. S. Marvel and V. C. Sekera, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 366.

Tautomerism of 5a,11a-Dehydro-7-chlorotetracycline. Preparation of 5-Alkoxy-7-chloroanhydrotetracyclines

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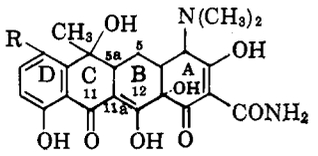
In 1958 McCormick and co-workers[1] reported the isolation of a microbial metabolite, which was shown to be a dehydro analog of the antibiotic chlorotetracycline[2,3] (**1a**). This metabolite was produced by a blocked mutant of *Streptomyces aureofaciens*. On

(1) J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **80**, 5572 (1958).

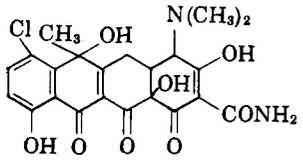
(2) B. M. Duggar, U. S. Patent, 2,482,055 (1949).

(3) 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).



1a, R = Cl
b, R = H

further fermentation[4] with a normal *S. aureofaciens* strain the metabolite was reduced to chlorotetracycline. It was thus suggested[4] that the dehydro analog was a biogenetic intermediate. The structure 7-chloro-5a,-11a-dehydrotetracycline (**2**) was assigned on the basis of the following observations. (i) The substance could be hydrogenated to a mixture of tetracycline (**1b**) and



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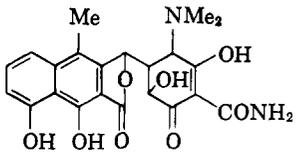
its C-5a epimer. (ii) Ultraviolet absorption changes were noted in the chromophore attributable[3] to the BCD ring system. (iii) An unspecified form of the metabolite showed infrared absorption at 5.8 μ. These early data define C-5a as a center of unsaturation. The present paper describes additional observations on this metabolite which demonstrate that C-5 may act as an unsaturated center. Of particular interest is a new synthetic route to 5-oxygenated tetracycline analogs.

In our hands, the dehydrochlorotetracycline metabolite was initially isolated as a yellow crystalline hydrochloride that showed no indication of carbonyl absorption below 5.9 μ (potassium bromide). Analytical, optical rotation, and ultraviolet absorption data on these crystals were similar to those reported earlier.[1,5] When the ultraviolet spectrum of the metabolite was determined in the presence of magnesium chloride, a strong shift was noted in the region above 350 mμ—a phenomenon previously shown by extensive model studies[6] to involve complex formation with an enolizable 11,12-dicarbonyl in the tetracycline series. This, as well as the observed pK_a's of the dehydro compound (3.3, 5.0, 9.6) suggest that its principal chromophore is a readily enolizable 11,12-diketone.

Strong acid treatment clearly implicated the 5-position as a reactive center in the metabolite. Thus, boiling the compound in aqueous hydrochloric acid resulted in a mixture of substances with ultraviolet absorption strongly reminiscent of the apoterramycins[7] (**3**, acid degradation products of 5-hydroxytetracycline).



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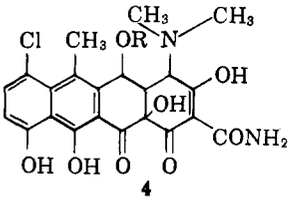
(4) J. R. D. McCormick, N. O. Sjolander, P. A. Miller, V. Hirsch, N. A. Arnold, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **80**, 6460 (1958).

(5) A comparison sample of the McCormick metabolite was kindly supplied by Dr. B. L. Hutchings of the American Cyanamid Co.

(6) *Cf.* L. H. Conover, *Chem. Soc.* (London) (Spec. Publ.), 48 (1956).

Treatment with dry hydrogen chloride in various alcohols resulted in a series of heretofore unknown tetracycline derivatives—the 5-alkoxyanhydrochlorotetracyclines[8] (**4**). These substances show ultraviolet absorption and the antimicrobial properties similar to



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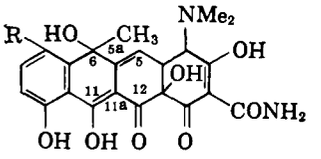
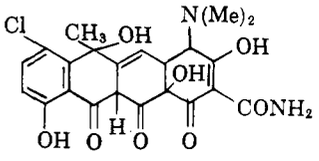
those of anhydrotetracycline.[9] This transformation is consistent with a starting intermediate of structure **5** which, by a simple allylic rearrangement, could generate the necessary C-5 carbonium ion.



5, R = Cl
6, R = H

The tautomeric nature of dehydrochlorotetracycline also was demonstrated by the isolation of two different crystalline tautomers of the amphoteric metabolite. One form, from water, shows no resolvable carbonyl absorption below 6 μ (potassium bromide or dioxane). When this form was warmed in chloroform, it was converted to a *ketonic tautomer*, λ_{max} 5.83 μ (chloroform or potassium bromide).

Because of the infrared spectrum it is attractive to assign the double bond in the ketonic tautomer of **5** to the 5,5a-position (*cf.* **7**) rather than the previously proposed 5a,11a-position.[10] Recently, Scott and Bedford have drawn similar conclusions[11] as to the position of the double bond in dehydroaureomycin. However, the n.m.r. spectrum of the ketonic tautomer in deuteriotetrahydrofuran shows no absorption in the region of 3.3–6.0 τ, the position expected for nonconjugated olefinic protons, and provides no evidence for a 5,5a-isomer in this solvent.



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The presence of a reactive center at 5a in the metabolite **5** together with several other recent observations[4,12]

(7) 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).

(8) The stereochemistry at C-5 in these compounds has not been determined.

(9) C. W. Waller, U. S. Patent 2,744,932 (1956).

(10) *Cf.* R. B. Turner and D. M. Voitle, *J. Am. Chem. Soc.*, **73**, 1403 (1951), for a detailed study of α,β- and β,γ-unsaturated forms of the model system 1-acetyl-2-methylcyclohexenone.

(11) A. I. Scott and C. T. Bedford, *ibid.*, **84**, 2271 (1962).

(12) (a) D. Pereman, L. J. Heuser, J. D. Dutcher, J. M. Barrett, and J. A. Boska, *J. Bacteriol.*, **80**, (3), 419 (1960); (b) J. R. D. McCormick, P. A. Miller, S. Johnson, N. Arnold, and N. O. Sjolander, *J. Am. Chem. Soc.*, **84**, 3023 (1962).

leads to the speculation that a common biogenetic intermediate such as **6** may explain the observed formation of both tetracycline and 5-hydroxytetracycline by various *Streptomyces* strains. Such an intermediate as **6** might be visualized as undergoing either fermentative reduction or hydration (peroxidation–reduction) in the final step of biogenesis. We feel this route to be an attractive possibility, although the conversion of 5a,6-anhydro-5-hydroxytetracycline to 5-hydroxytetracycline has been accomplished by an *S. aureofaciens* strain.[12b]

Experimental

Isolation of Dehydrochlorotetracycline.—A *Streptomyces aureofaciens* mutant was grown on a medium similar to that used for the production of chlorotetracycline.[2] This medium included 75 g. of cornstarch, 25 g. of corn steep liquor, and 10 ml. of soybean oil per liter, plus the usual organic salts and calcium carbonate. After a 2-day inoculum incubation, a 250-gal. tank was run for 5 days at 26° with 12 cu. ft. of sterile air/hr./gal.; terminal pH, 7.3.

The broth, 157 gal., was adjusted to pH 2 with sulfuric acid; 75 lb. of Supercel was added, filtered, and filtrate adjusted to pH 8.5 with sodium hydroxide. The precipitate which formed was filtered on a press and washed with water to yield 16 kg. of wet cake. This cake contained small amounts of chlortetracycline as well as dehydroaureomycin, as a metal complex. The wet broth precipitate (16 kg.) was slurried in isopropyl alcohol (17 l.). After the slurry was acidified to pH 1.9 with concentrated hydrochloric acid, sodium chloride (3 kg.), butanol (34 l.), and Supercel (650 g.) were added. The mixture was filtered and the phases were separated. To the aqueous phase was added the filtration residue, isopropyl alcohol (8.5 l.), butanol (17 l.), and sufficient concentrated hydrochloric acid to adjust the pH to 1.5. After filtration the phases were separated. The combined organic phases were concentrated under reduced pressure to a volume of 15 l. and filtered from precipitated solids. To the filtrate were added 0.01 *N* hydrochloric acid (3 l.) and hexane (34 l.). After separation of the phases the organic phase was extracted twice with 0.01 *N* hydrochloric acid (1.5 l.). The combined acid extracts were freeze dried; residue, 416 g., was dissolved in methanol (2.5 l.) and filtered from insolubles. On addition of ethyl acetate (1.1 l.) crystals formed on cooling and standing for 2 days. The crystals were collected and washed with ethanol; yield, 107 g. A small sample was recrystallized from methanol–ethanol. Its infrared spectrum was identical with that of an authentic sample.[5]

Anal. Calcd. for $C_{22}H_{22}N_2Cl_2O_8$: C, 51.48; H, 4.32; N, 5.46; Cl, 13.81. Found: C, 51.28; H, 4.58; N, 4.99; Cl, 13.05.

The ultraviolet absorption spectrum in methanol–hydrochloric acid showed λ_{max} 254, 383 mμ (log ε 4.3, 3.6), while in methanol–sodium hydroxide the absorption was shifted to 247, 260 (sh), 424 mμ (log ε 4.3, 4.3, 4.6), and in methanol–magnesium chloride to 238, 268, 410 mμ (log ε 4.47, 4.4, 4.04). The optical rotation was determined in 0.67% solution in 0.03 *N* hydrochloric acid. The value was found to change with time as follows: $[\alpha]^{25}D$ +6.8° (15 min.); +12.5° (105 min.); +1° (19 hr.). The pK_a's of another sample of dehydrochlorotetracycline hydrochloride were determined in 0.1 *N* potassium chloride solution.[13] The values found were *ca.* 3.3; 4.98 ± 0.10; 9.64 ± 0.10; neut. equiv., 528 (calcd. 512). Qualitative observations of ultraviolet absorption *vs.* pH taken in aqueous solution (*c* 4.10^{-5} *M*) indicate that an anion forms with increasing pH in the range pH 3.1 (λ_{max} 375 mμ) to pH 6.0 (λ_{max} 405 mμ); the pH at the inflection point is *ca.* 4.6. This furnished a qualitative corroboration of the pK_a 2 value.

Amphoteric Dehydrochlorotetracycline.—One gram of dehydrochlorotetracycline hydrochloride was slurried in 50 ml. of water and adjusted to pH 7.0. The solution so formed was filtered, then adjusted to pH 3.0. A light yellow crystalline solid separated (0.7 g.), which showed only a trace of 5.8-μ infrared absorption in either potassium bromide pellet or dioxane solution. This material (0.65 g.) was boiled in chloroform for 4.5 hr., filtered from a trace of insoluble residue, the filtrate reduced to dryness and recrystallized from 25 ml. of chloroform containing 4 ml. of hexane. The crystals so obtained were dried at 60° (0.01 mm.) for 18 hr. They showed very strong infrared absorption at 5.83 μ either in potassium bromide pellet or in chloroform solution. The crystals decomposed indefinitely above 181°.

Anal. Calcd. for $C_{22}H_{21}N_2O_8Cl$: C, 55.41; H, 4.44; N, 5.88. Found: C, 54.93; H, 4.65; N, 5.71; λ_{max} ($CHCl_3$) 366 mμ (log ε 3.58).

The n.m.r. spectrum of the ketonic tautomer was measured in octadeuteriotetrahydrofuran. No signal was observed in the region of 3.3–6 τ. The aromatic protons were indicated as doublets at 2.45 and 3.1 τ. The infrared spectrum of the solution used for n.m.r. measurements showed a strong peak at 5.82 μ.

Acid Degradation of Dehydrochlorotetracycline.—Ten milligrams of dehydrochlorotetracycline hydrochloride was dissolved in 5 ml. 1 *N* hydrochloric acid and heated at 95° for 24 min. An amorphous precipitate formed which showed an ultraviolet absorption spectrum of λ_{max} 248, 320, 373 mμ, reminiscent of apoterramycin.

5-Methoxy-7-chloroanhydrotetracyclines.—A 0.5% solution of 7-chlorodehydrotetracycline hydrochloride was heated under reflux in 0.1 *N* methanolic hydrochloric acid for 17 hr. The solution was concentrated under reduced pressure, filtered, and the crude product precipitated with ethyl acetate. Further purification was achieved by recrystallization from ethyl acetate. Paper chromatography showed the product to be homogeneous and different from anhydrochlorotetracycline or anhydrotetracycline; λ_{max} (MeOH·HCl) 229, 272, 337, 433 mμ; log ε 4.37, 4.58, 3.45, 3.86; $[\alpha]^{25}D$ −229° (*c* 0.2, MeOH).

Anal. Calcd. for $C_{23}H_{24}N_2O_8Cl_2 \cdot {}^1/_2H_2O$: C, 51.50; H, 4.70; N, 5.23; Cl, 13.18; OCH_3, 5.79; C-CH_3, 2.80. Found: C, 51.29; H, 4.81; N, 5.28; Cl, 15.10; OCH_3, 6.58; C-CH_3, 2.97.

5-Ethoxy-7-chloroanhydrotetracycline.—This compound was prepared by the procedure described before, substituting ethanol for methanol; λ_{max} (MeOH·HCl) 229, 274, 335, 438 mμ; log ε 4.39, 4.55, 3.55, 3.83; $[\alpha]^{25}D$ −181° (*c* 0.2, MeOH).

Anal. Calcd. for $C_{24}H_{26}N_2O_8Cl_2$: C, 53.24; H, 4.84; N, 5.18; OC_2H_5, 8.32. Found: C, 53.71; H, 5.25; N, 4.85; OC_2H_5, 9.1.

Other 5-alkoxy-7-chloroanhydrotetracyclines, including the isopropoxy and benzyloxy derivatives, were prepared in a similar manner by heating the reaction mixture under reflux or to 100° for several hours and were characterized by paper chromatography and by their ultraviolet absorption spectra.

Acknowledgment.—We are indebted to Mr. E. Tynan and Dr. F. W. Tanner, Jr., for the fermentation of the dehydroaureomycin. Drs. R. L. Wagner, Jr., Kotaro Murai, and their associates provided the physical measurement data.

(13) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings, and W. A. Lazier, *J. Am. Chem. Soc.*, **73**, 4211 (1951).

Quinazolines and 1,4-Benzodiazepines. XI.[1] Synthesis and Transformations of 7-Chloro-2,3-dihydro(and 2,3,4,5-tetrahydro)-5-phenyl-1*H*-1,4-benzodiazepine[2]

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Received March 20, 1963

Our interest in benzodiazepine derivatives prompted us to study methods for the synthesis of 2,3-dihydro-5-

(1) Paper X, L. H. Sternbach, R. Ian Fryer, O. Keller, W. Metlesics, G. Sach, and N. Steiger, *J. Med. Chem.*, **6**, (3) 261 (1963).

(2) The material contained in this paper and the synthesis of analogs of IV, bearing in position 7, a hydrogen or bromine atom, a methyl, carboxy, or carbomethoxy group, are described in the Hoffmann-La Roche Belgian Patent 620773 (Derwent Abstracts of Feb. 8, 1963). This application also contains derivatives of IV bearing an additional substituent in the phenyl ring (2′-F, Cl, OCH_3) and analogs of XIc bearing an amino, dimethylamino, or cyano group in position 7. Part of this material will be described in a further communication.