chloride and the filtrate was diluted with water (1 l.) and concentrated sufficiently at 20 mm. to remove most of the organic solvents. The crude crystals (72 g.) of ethylidenedimercuric chloride were collected and rinsed with acetone. Further dilution of the mother liquor to 3 l. yielded another 35 g. of dubious quality, and extraction of the mercurous chloride precipitate with dimethyl sulfoxide followed by dilution with acetone and water yielded an additional 17 g. of fairly pure crystals. (The mercurous chloride residue was 65 g.) Recrystallization of all the crude fractions from 375 ml. of hot dimethylformamide, with filtration to remove about 15 g. of insoluble material, yielded 55.6 g. of **8** in the first crop and 15.4 g. more after dilution of the mother liquor with 300 ml. of water, both of m.p. 217–221° dec., 28% yield. The analytical sample was recrystallized three times from dimethylformamide, washed freely with acetone, and dried at 55° (0.05 mm.), m.p. 219–221° dec.

Anal. Calcd. for $C_2H_4Hg_2Cl_2$: C, 4.80; H, 0.81; Cl, 14.18; Hg, 80.22. Found: C, 4.79; H, 0.97; Cl, 13.93; Hg, 80.42.

Preparation of Tetracyclines by Photooxidation of Anhydrotetracyclines

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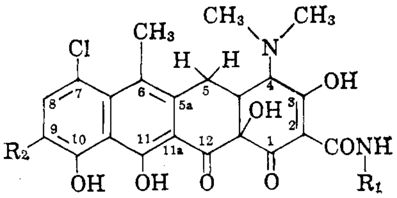
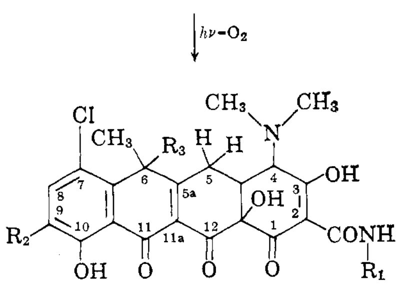
The preparation of N-*t*-butyl-, 9-N-di-*t*-butyl-, and 7-chloro-9-N-di-*t*-butyltetracycline (IIIb–d) by photooxidation of the corresponding 7-chloroanhydrotetracycline derivatives Ib and c and subsequent reduction is described. The oxidation is catalyzed by 3,4-benzpyrene and leads to the crystalline 5a,11a-dehydro derivatives IIb and c.

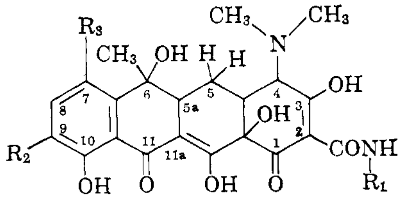
Possibilities for chemical modifications of the tetracycline molecule (IIIa) always have been limited severely by the presence of the acid-labile, C-6 benzylic hydroxyl function. For this reason many reactions in the past were carried out employing the C-6 deoxy derivatives of this important class of antibiotics.[1] A new important route to tetracycline derivatives containing the C-6 hydroxyl function became available when Scott and Bedford discovered the conversion by photooxidation of 7-chloroanhydrotetracycline (Ia) to 7-chloro-6-deoxy-6-peroxydehydrotetracycline[2] (IIa). Reduction of the latter compound yields tetracycline (IIIa). We wish to report on the application of this procedure to the preparation of N-*t*-butyl- and 9-N-di-*t*-butyltetracyclines (IIIb–d) and on some observations made during these studies.



Ia, $R_1 = R_2 = H$
b, $R_1 = t$-butyl; $R_2 = H$
c, $R_1 = R_2 = t$-butyl



IIa, $R_1 = R_2 = H$; $R_3 = OOH$
b, $R_1 = t$-butyl; $R_2 = H$; $R_3 = OOH$
c, $R_1 = R_2 = t$-butyl; $R_3 = OOH$
d, $R_1 = R_2 = H$; $R_3 = OH$
e, $R_1 = R_2 = t$-butyl; $R_3 = OH$



IIIa, $R_1 = R_2 = R_3 = H$
b, $R_1 = t$-butyl; $R_2 = R_3 = H$
c, $R_1 = R_2 = t$-butyl; $R_3 = H$
d, $R_1 = R_2 = t$-butyl; $R_3 = Cl$

For our experiments we employed mercury vapor lamps with a Pyrex glass filter. Irradiation and oxygenation of a benzene solution of 7-chloroanhydrotetracycline (Ia) yielded the product IIa which crystallized spontaneously from the reaction mixture, as has been described already.[2] However the addition of small quantities of 3,4-benzpyrene greatly accelerated this process. The effect of the catalyst was especially pronounced with weak radiation sources (Table I). In general, a yield of 80% could be obtained in relatively short time without recycling.

TABLE I

YIELDS OF PHOTOOXIDATION PRODUCTS UNDER VARIOUS CONDITIONS

Lamp, w.	Irradiation, hr.	Yield, %
450	4	42
450	4[a]	78
100	5	0
100	5[a]	28

[a] With catalyst.

Crystallization of the product during the reaction was highly desirable to avoid decomposition of the newly formed compound by further irradiation. Since the alkylated dehydrotetracycline derivatives are highly soluble in benzene, different solvent systems were employed for these compounds to ensure adequate yields. Thus, cyclohexane appeared to be the solvent of choice for the oxidation of 9-N-di-*t*-butyl-7-chloroanhydrotetracycline (Ic). For N-*t*-butyl-7-chloroanhydrotetracycline (Ib) a mixture of benzene and cyclohexane was employed. Under these conditions the desired prod-

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[1] Many independent studies have appeared. For leading references, *cf.* (a) C. R. Stephens, J. J. Berreboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963); (b) J. J. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *J. Med. Chem.*, **6**, 405 (1963).

[2] A. I. Scott and C. T. Bedford, *J. Am. Chem. Soc.*, **84**, 2271 (1962).

ucts also crystallized directly from the reaction mixture.

Strong infrared absorption at 5.8 μ indicated that the 6-peroxydehydro compounds, IIa, b, and c, crystallized in their ketonic forms. Like the ketonic tautomer of 7-chlorodehydrotetracycline (IId)[3] these products showed no signals in the n.m.r. spectrum for olefinic protons between τ 3.1 and 6.5. Therefore the double bond assumed the C-5a–C-11a rather than the previously suggested C-5–C-5a position.[2] It is possible, however, that in solution an equilibrium is achieved between these ketonic tautomers *via* their common enol whose formation on standing in solution is indicated by changes in the ultraviolet absorption spectrum.

At least two mechanisms can be envisioned for the photooxidation reaction. (a) Hydrogen is abstracted from C-5 with shift of the double bond from C-5a–C-6 to C-5–C-5a to form a β,γ-unsaturated enol. Ketonization would occur subsequently with addition of a proton at C-5. (b) Oxygen adds across the C-ring and the addition product breaks down directly into the 5a,11a-unsaturated compound without any involvement of C-5.

When 7-chloroanhydrotetracycline (Ia) was irradiated whose labile (NH, OH) protons had been exchanged for deuterium, the crystalline, 7-chloro-6-deoxy-6-peroxydehydrotetracycline (IIa) obtained did not contain deuterium at C-5, as shown by n.m.r. spectroscopy. This experiment, therefore, did not indicate participation of C-5 in the oxidation process.

Reduction of the dehydro compounds II was achieved catalytically. It appeared to proceed with greater ease at a pH (4–7) that permits rapid enolization, *i.e.* shift of the double bond to the more accessible C-5–C-5a position. Under the conditions used chlorine was hydrogenolized in compounds unsubstituted at C-9 at a faster rate than in the 9-N-di-*t*-butyl derivatives which yielded both the 7-chloro- as well as the 7-deschloro-9-N-di-*t*-butyltetracyclines.

Experimental

The photooxidation experiments were carried out in a cylindrical Pyrex vessel which was fitted at its lower end with a sintered-glass filter and a stopcock arrangement that permitted gas to be blown into the vessel through the perforated bottom. The upper end of the vessel carried a ground-glass joint into which a double-walled quartz immersion well, as supplied by Hanovia Lamp Division, was fitted. An additional opening at the upper end permitted gas to escape. As radiation source, either a 100- or a 450-w. Hanovia laboratory photochemical lamp was lowered into the immersion well, together with a Pyrex filler sleeve. Oxygen was blown through the solution at a rate of 200 ml./min. and the temperature was maintained by cooling at about 30°.

7-Chloro-6-deoxy-6-peroxydehydrotetracycline (IIa).—7-Chloroanhydrotetracycline (Ia, 5 g.) was dissolved in benzene (1400 ml.). After addition of 3,4-benzpyrene (25 mg.) the solution was irradiated (450-w. lamp) and oxygenated for 5 hr. The solution was seeded after the first hr. The crystalline product IIa (4.2 g.) was collected at the end of the experiment. Recrystallization from dioxane–benzene furnished an analytical sample: λ_{max}^{KBr} 5.83 μ; $\lambda_{max}^{MeOH-HCl}$ 249 and 370 mμ (log ϵ 4.31 and 3.57); $\lambda_{max}^{MeOH-NaOH}$ 243, 265, and 407 mμ (log ϵ 4.37, 4.31, and 3.90). On standing in MeOH–HCl the ultraviolet absorption slowly shifted from 370 to 384 mμ. The n.m.r. spectrum in octadeuteriotetrahydrofuran shows no signal in the region of τ 3.3–6.0. The signals for the aromatic protons appeared at τ 2.45 and 2.03 as doublets.

Anal. Calcd. for $C_{22}H_{21}ClN_2O_9$: C, 53.64; H, 4.30; N, 5.69. Found: C, 53.90; H, 4.39; N, 5.38.

To demonstrate the efficacy of 3,4-benzpyrene as a catalyst the above reaction was carried out in the absence as well as presence of 3,4-benzpyrene with the 450- and 100-w. lamp. In all instances the solutions were seeded frequently. The results are shown in Table I.

Deuterated 7-chloro-6-deoxy-6-peroxydehydrotetracycline was prepared in the following manner. 7-Chloroanhydrotetracycline (Ia, 3.5 g.) was dissolved in benzene (2 l.). After distillation of 200 ml. of solvent, CH_3OD (10 g.) was added. More solvent (200 ml.) was distilled and again CH_3OD (10 g.) was added. This operation was repeated twice more and finally 200 ml. of solvent was distilled. Part (800 ml.) of the remaining solution was irradiated and oxygenated under the usual conditions. The crystalline product obtained did not differ in its n.m.r. spectrum from the undeuterated compound IIa with the exception that the signals below τ 2.2, normally associated with N–H and O–H protons, were absent.

N-*t*-Butyl-7-chloro-6-deoxy-6-peroxydehydrotetracycline (IIb).—N-*t*-Butyl-7-chloroanhydrotetracycline[4] (Ib, 6.3 g.) in benzene (200 ml.) and cyclohexane (800 ml.) was irradiated and oxygenated in the presence of 3,4-benzpyrene (25 mg.). After 1 hr. the reaction mixture was seeded and cyclohexane was added (100 ml.). Cyclohexane was added three times more at 1-hr. intervals in quantities of 100 ml. The crystalline product was collected after 5 hr. (2.85 g.). Recrystallization from hexane yielded an analytical sample: λ_{max}^{KBr} 5.83 μ; $\lambda_{max}^{MeOH-HCl}$ 253 and 375 mμ (log ϵ 4.31 and 3.60). The n.m.r. spectrum ($CDCl_3$) showed no signal between τ 3.2 and 6.5.

Anal. Calcd. for $C_{26}H_{29}ClN_2O_9$: C, 56.88; H, 5.32; N, 5.10. Found: C, 56.65; H, 5.38; N, 4.80.

9-N-Di-*t*-Butyl-7-chloro-6-deoxy-6-peroxydehydrotetracycline (IIc).—9-N-Di-*t*-butyl-7-chloroanhydrotetracycline[4] (Ic, 10 g.) was irradiated (450-w. lamp) and oxygenated in cyclohexane (800 ml.) in the presence of 3,4-benzpyrene (25 mg.) for 5 hr. The crystalline product IIc (4.5 g.) was collected, and, after cleaning the immersion well from precipitated product, irradiation was continued for another 2 hr. Again the crystalline product was collected (1.85 g.). Additional product (2.1 g.) could be obtained by further irradiation of the concentrated mother liquor. An analytical sample, containing 0.5 equiv. of solvent, was obtained by recrystallization from cyclohexane: $\lambda_{max}^{KBr\ or\ CHCl}$ 5.83 μ; $\lambda_{max}^{MeOH-HCl}$ 260 and 375 mμ (log ϵ 4.32 and 3.60); $\lambda_{max}^{MeOH-NaOH}$ 242, 272, and 412 (log ϵ 4.37, 4.30, and 3.91). The n.m.r. spectrum ($CDCl_3$) showed no signal between τ 3 and 6.2 but confirmed the presence of 0.5 mole of cyclohexane.

Anal. Calcd. for $C_{30}H_{37}ClN_2O_9 \cdot 0.5C_6H_{12}$: C, 61.24; H, 6.70; N, 4.33. Found: C, 61.26, 61.35; H, 6.66, 6.82; N, 4.44, 4.18.

N-*t*-Butyltetracycline (IIIb).—N-*t*-Butyl-7-chloro-6-deoxy-6-peroxydehydrotetracycline (IIb, 1 g.) was hydrogenated in ethanol (80 ml.) and a 2% aqueous monopotassium phosphate solution (20 ml.) over palladium black (600 mg.) at room temperature at 50 p.s.i for 3 hr. After filtration, water (200 ml.) was added and the solution was extracted with chloroform. The organic extract was evaporated to dryness and the residue was dissolved in 0.01 *N* hydrochloric acid (100 ml.). After extraction with ether, the aqueous phase was adjusted to pH 5.1 with a disodium phosphate solution. The aqueous phase was again extracted with ether. Evaporation of the latter phase yielded crude N-*t*-butyltetracycline (250 mg.). An analytical sample was obtained by precipitating the compound from ether solution with 1 equiv. of alcoholic hydrochloric acid: $\lambda_{max}^{MeOH-HCl}$ 267 and 359 mμ (log ϵ 4.34 and 4.1); $\lambda_{max}^{MeOH-NaOH}$ 240 and 265 sh and 378 mμ (log ϵ 4.31, 4.21, and 4.16).

Anal. Calcd. for $C_{26}H_{32}N_2O_8 \cdot HCl \cdot C_2H_5OH$: C, 57.58; H, 6.74; N, 4.80; C_2H_5O 7.76. Found: C, 57.64; H, 6.49; N, 5.14; C_2H_5O, 6.21.

7-Chloro-9-N-di-*t*-butyldehydrotetracycline (IIe).—7-Chloro-9-N-di-*t*-butyl-6-deoxy-6-peroxydehydrotetracycline (IIc, 2 g.) was hydrogenated in ethanol (125 ml.) and benzene (25 ml.) over palladium black (1 g.) at room temperature at 50 p.s.i. for 10 min. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue was dissolved in ether, and toluenesulfonic acid (600 mg.) dissolved in ether was added slowly. The resulting precipitate was filtered and crystal-

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[3] M. Schach von Wittenau, F. A. Hochstein, and C. R. Stephens, *J. Org. Chem.*, **28**, 2454 (1963).

[4] C. R. Stephens, U. S. Patent 3,028,409 (April 3, 1962); *cf.* ref. 1a for an analogous preparation.

lized from methanol (5 ml.). Recrystallization from methanol–ether yielded an analytical sample (780 mg.): $\lambda_{\max}^{\text{KBr}}$ 5.83 μ; $\lambda_{\max}^{\text{MeOH-HCl}}$ 255 and 395 mμ (log ϵ 4.37 and 3.80): $\lambda_{\max}^{\text{MeOH-NaOH}}$ 245, 345, and 420 mμ (log ϵ 4.41, 3.70, and 4.15).

Anal. Calcd. for $C_{30}H_{37}ClN_2O_8 \cdot C_7H_8O_3S$: C, 58.37; H, 5.96; Cl, 4.66; N, 3.68. Found: C, 58.38; H, 5.95; Cl, 4.67; N, 3.52.

7-Chloro-9-N-di-*t*-butyltetracycline (IIId).—The above compound IIe (720 mg.) was hydrogenated in ethanol (50 ml.) and 0.067 *M* pH 7 phosphate buffer (45 ml.) over palladium black (500 mg.) at 50 p.s.i. at room temperature for 30 min. The reaction mixture was filtered and added to a mixture of ethyl acetate, cyclohexane, and water. The organic layer was washed with water, dried, and evaporated. The residue (550 mg.) was dissolved in ether and added slowly to a stirred solution of *p*-toluenesulfonic acid (175 mg.) in ether (150 ml.). The resulting precipitate (218 mg.) was filtered. The filtrate was concentrated to yield pure product (45 mg.): $\lambda_{\max}^{\text{MeOH-HCl}}$ 258, 342, and 373 mμ (log ϵ 4.32, 3.89, and 3.96); $\lambda_{\max}^{\text{MeOH-NaOH}}$ 243, 270, and 391 mμ (log ϵ 4.33, 4.23, and 4.11).

Anal. Calcd. for $C_{30}H_{39}ClN_2O_8 \cdot C_7H_8O_3S$: C, 58.22; H, 6.21; Cl, 4.65. Found: C, 58.46; H, 6.32, Cl, 3.42.

9-N-Di-*t*-butyltetracycline (IIIc).—7-Chloro-9-N-di-*t*-butyl-6-deoxy-6-peroxydehydrotetracycline (IIc, 1.9 g.) was hydrogenated in ethanol (125 ml.) and benzene (25 ml.) over palladium black (1 g.) at room temperature at 50 p.s.i. for 18 hr. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue was distributed between ether and pH 4.5 phosphate buffer and the ether phase was evaporated to dryness. The material obtained was again hydrogenated in ethanol (100 ml.) over palladium black (1 g.) for 3 hr. After filtration and evaporation to dryness the residue was distributed between ether and pH 4.5 buffer. The ether phase was dried and evaporated to yield the crude product (1.48 g.). This was dissolved in cyclohexane (400 ml.), and *p*-toluenesulfonic acid (445 mg.) dissolved in ethanol (2 ml.) was added. The resulting precipitate was purified by reprecipitation from methanol–ether: $\lambda_{\max}^{\text{MeOH-HCl}}$ 271, 341, and 366 mμ (log ϵ 4.35, 4.06, and 4.07); $\lambda_{\max}^{\text{MeOH-NaOH}}$ 269 and 380 mμ (log ϵ 4.26 and 4.23).

Anal. Calcd. for $C_{30}H_{40}N_2O_8 \cdot C_7H_8SO_3$: C, 60.97; H, 6.64; N, 3.84. Found: C, 60.53; H, 6.54; N, 3.75.

Anhydrotetracycline (1 g.) was irradiated and oxygenated in benzene solution (400 ml.) in the presence of 3,4-benzpyrene (15 mg.) for 7 hr. The solution was extracted with 0.01 *N* hydrochloric acid (100 ml.) and water (50 ml.) and the combined aqueous phases were hydrogenated at room temperature at 50 p.s.i. for 4 hr. over 5% palladium on carbon (500 mg.). The filtered solution contained, as shown by a bio-plated paper chromatogram, tetracycline as the only bio-active compound. The reaction mixture was freeze-dried to yield a crude product (486 mg.) containing anhydrotetracycline as well as tetracycline as indicated by a paper chromatogram. The crude product showed activity of 120 μ/mg. of tetracycline standard (1 mg. = 1000 μ), *K. pneumoniae* assay.

Dedimethylamino-7-chloroanhydrotetracycline also underwent the photooxidation process, as indicated during the reaction by the characteristic shift of ultraviolet absorption of the reaction mixture from an anhydro- to a dehydrotetracycline type.

Restricted Internal Reorientation in Large-Ring N-Methyllactams as Evidenced by Nuclear Magnetic Resonance[1]

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The nuclear magnetic resonance spectra of N-methylcaprolactam (I), N-methylcapryllactam (II), and N-methyllauryllactam (III) have been measured at various temperatures. The nine-membered lactam II displays singlet N-methyl absorption at temperatures as low as −30°, while the thirteen-membered lactam III possesses an N-methyl doublet at room temperature (δ_{AB} = 7 c.p.s.); coalescence occurs at 60°. Observation of the temperature-dependent N-methyl doublet in this latter case is considered to arise from *cis* and *trans* isomers about the amido group. The nature of the *cis* and *trans* isomers in large-membered N-methyl lactams is discussed as well as the mechanisms for interconversion. N-Methylation of lactams with sodium hydride and methyl iodide is described.

N.m.r. has proved extremely useful for the study of hindered internal rotation such as that occurring about the C–N bond in various N-methylamides.[2] Observation of a temperature-dependent N-methyl doublet signal corresponds to exchanging N-methyl rotamers which interconvert at a rate slower than the chemical shift difference between N-methyl *cis* and N-methyl *trans* to oxygen.[2] The mean lifetime in each state, $\tau_A\tau_B$, is therefore large compared with the inverse frequency separation $(\omega HA - \omega HB)^{-1}$. Application of this type of measurement to the cyclic analogs, N-methyl lactams, is of interest in order to determine the possible existence of *cis–trans* amido group exchange in large rings and to learn the range of ring sizes in which *trans* isomers may occur. Furthermore, in the case of coexisting *cis–trans* isomers, measurement of the relative absorption intensity of CH_3N *cis* to CO *vs.* CH_3N *trans* to CO should indicate the mean populations in each state, thus allowing the calculation of an equilibrium constant for a given system at constant conditions of temperature, solvent, and concentration. We wish to report experimental findings on these points.

Table I lists the chemical shifts for the ring CH_2, CH_2CO, CH_2–N, and CH_3–N for N-methylcaprolactam (I, seven-membered), N-methylcapryllactam (II, nine-membered), and N-methyllauryllactam (III thirteen-membered). The positions of the proton resonance are based upon abundant analogy; spectra have been reported for pyrrolidone, caprolactam, and N-methylpyrrolidone.[3] The most striking feature of these results is the doublet N-methyl resonance shown in the spectrum of the thirteen-membered example (Fig. 1). At 30° the peaks are separated by 7.1 c.p.s. Warming progressively to a temperature of 60° leads to coalescence of the two N-methyl components into a single peak (Fig. 1). Also the sharpness of both α-methylenes is

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[1] Acknowledgment is made to the National Institutes of Health (GM11595-01) for support of this research.

[2] J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 218–365.

[3] N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "High Resolution N.M.R. Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, entries 68 and 116, respectively. Proton resonance values for caprolactam have been reported by G. Van Dyke Tiers, "Characteristic N.M.R. Shielding Values (Spectral Positions) for Hydrogen in Organic Compounds," Minnesota Mining and Manufacturing Co., St. Paul, Minn., 1958, pp. 16, 18.