expected to increase biological activity. The extension of the peptide chain increases the biological potency of the hexapeptide by about 10 fold (see Table I). Thus, the contribution aspartic acid and arginine make to the stability of the conformation of angiotensin is important to its biological activity.

The rate of the myotropic and pressor responses and the duration of these responses to N-(poly-O-acetyl-L-seryl)-angiotensin II were identical to those produced by angiotensin II. Since the large polymer would probably not pass into a cell readily, this indicates these peptides are causing a myotropic response by acting on the cell membrane.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, A DIVISION OF AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

The 6-Deoxytetracyclines. III. Electrophilic and Nucleophilic Substitution[1]

BY JOSEPH J. HLAVKA, A. SCHNELLER, H. KRAZINSKI AND J. H. BOOTHE

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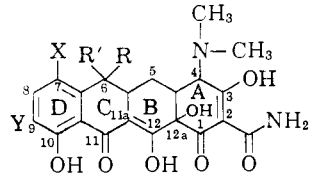
A series of electrophilic and nucleophilic substitutions upon the aromatic D ring of 6-deoxytetracycline and 6-demethyl-6-deoxytetracycline are described. The positions of entering groups are assigned on the basis of a novel method of tritium replacement. The relationship between structure and antibacterial activity is discussed.

The tetracycline molecule, I, has always presented a special problem to the organic chemist interested in the study of structure–activity relationships. The difficulty has been to devise chemical pathways which would bring about the necessary transformation yet preserve the rather complicated and sensitive B, C, D ring chromophore. The lability of the 6-hydroxyl group to both acid[2] and base[3] degradation prevented any substantial progress in this field. Moreover, the ease of epimerization[4] at the carbon atom at position 4 added to the problem of chemical instability under many reaction conditions.

The stability of the recently isolated 6-deoxytetracycline[5] (II) and 6-demethyl-6-deoxytetracycline[5] (III) permitted, for the first time, an ingress to this problem of chemical modification with retention of biological activity by a series of electrophilic substitutions under strongly acid conditions.[1] We now wish to report on an extension of this study using both electrophilic halogenation and nucleophilic displacement in the aromatic D ring.



I, R = CH_3, R′ = OH, X = Y = H
II, R = CH_3, R′ = X = Y = H
III, R = R′ = X = Y = H
IV,[6] R = R′ = Y = H, X = Cl
V, R = R′ = Y = H, X = Br
VI, R = R′ = Y = H, X = I
VII, R = CH_3, R′ = Y = H, X = Br
VIII, R = CH_3, R′ = Y = H, X = I
IX, R = CH_3, R′ = X = H, Y = NH_2
X, R = R′ = Y = H, X = NH_2
XI, R = R′ = X = H, Y = NH_2

XII, R = CH_3, R′ = X = H, Y = $-\overset{+}{N}\equiv N$

XIII, R = R′ = Y = H, X = $-\overset{+}{N}\equiv N$

XIV, R = R′ = X = H, Y = $-\overset{+}{N}\equiv N$

XV, R = CH_3, R′ = X = H, Y = $-\overset{-}{N}-\overset{+}{N}\equiv N$

XVI, R = R′ = Y = H, X = $-\overset{-}{N}-\overset{+}{N}\equiv N$

XVII, R = R′ = X = H, Y = $-\overset{-}{N}-\overset{+}{N}\equiv N$

XVIII, R = CH_3, R′ = X = H, Y = $-S-\overset{S}{\overset{\|}{C}}-OC_2H_5$

XIX, R = R′ = Y = H, X = $-S-\overset{S}{\overset{\|}{C}}-OC_2H_5$

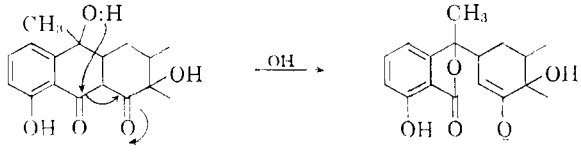
XX, R = R′ = X = H, Y = $-S-\overset{S}{\overset{\|}{C}}-OC_2H_5$

We have found that treatment of 6-demethyl-6-deoxytetracycline (III) with N-bromosuccinimide in concentrated sulfuric acid at 0° yielded a single monobromo-6-demethyl-6-deoxytetracycline, V.

(1) (a) A preliminary report of this material has been published in *J. Am. Chem. Soc.*, **82**, 1253 (1960); (b) Paper II of this series has been submitted to the *J. Med. Pharm. Chem.*; (c) *cf.* J. J. Beereboom, J. J. Ursprung, H. H. Rennhard and C. R. Stephens, *J. Am. Chem. Soc.*, **82**, 1003 (1960).

(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); C. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. Broschard and J. H. Williams, *ibid.*, **74**, 4981 (1952). Acid treatment of tetracycline results in a ready *trans* elimination of the 6-hydroxy group to yield anhydrotetracycline.

(3) *Ibid.*: Base treatment of tetracycline yields isotetracycline.

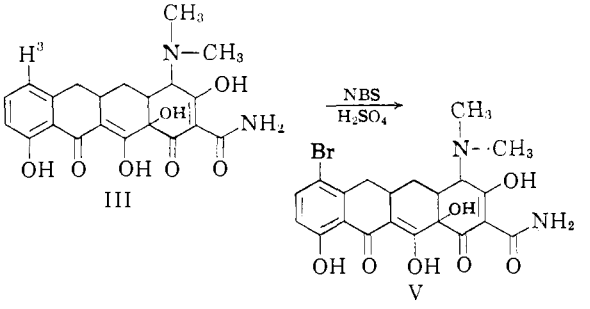


(4) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. E. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *ibid.*, **79**, 2849 (1957).

(5) (a) J. R. D. McCormick, E. R. Jensen, P. A. Miller and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960); (b) C. R. Stephens, *et al.*, *ibid.*, **80**, 5324 (1958).

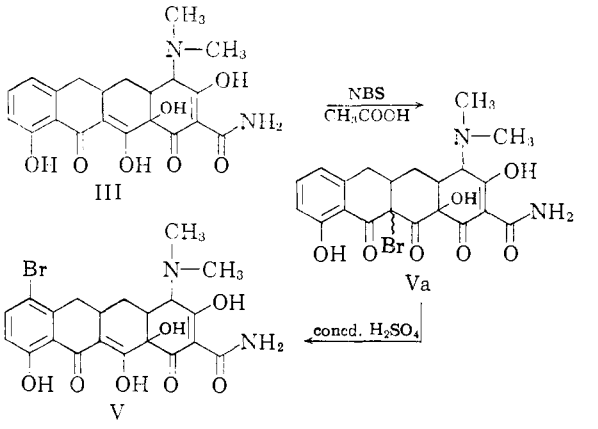
(6) J. R. D. McCormick and E. R. Jensen, German Patent 1,082,905, June 9, 1960.

Unequivocal proof for the assignment of the bromine atom to the 7-position was obtained by carrying out the halogenation on 6-demethyl-6-deoxytetracycline labeled with tritium in the 7-position.[7,8] Replacement of the tritium[9] by bromine provided convincing evidence for this structural assignment. In a similar manner, reaction of

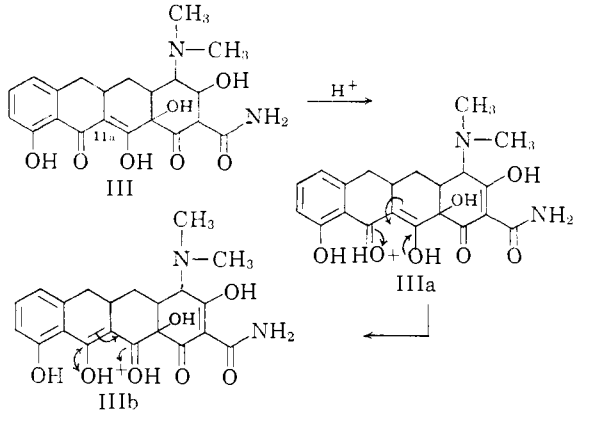


6 - demethyl - 6 - deoxytetracycline (III) with N-iodosuccinimide gave a previously unknown iodotetracycline (VI). Reaction of 6-deoxytetracycline (II) with either N-bromosuccinimide or N-iodosuccinimide yielded the corresponding 7-bromo (VII) and 7-iodo (VIII) derivatives, respectively. Some preliminary studies[10] on the preferential localization of 7-iodo-6-deoxytetracycline-I^{131} in tumor tissue suggest the use of this compound as a diagnostic aid in the location of cancerous growth.

Using less acidic conditions (*e.g.*, acetic acid), the reaction of 6-demethyl-6-deoxytetracycline (III) with N-bromosuccinimide gives exclusively 11a-bromo-6-demethyl-6-deoxytetracycline (Va) as indicated by loss of ultraviolet absorption in the 350 mμ region and a corresponding appearance of a new ketone stretching frequency at 1739 cm.$^{-1}$ in the infrared (due to the isolated carbonyl in the B ring). The tendency of N-halosuccinimides to react differently with a tetracycline depending upon

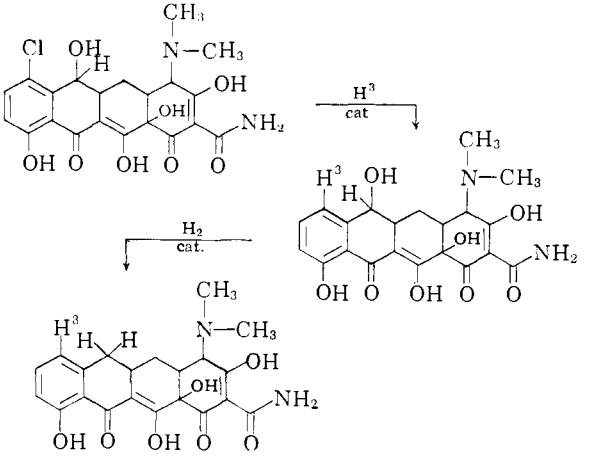


the acidity of the reaction medium suggests a special role for the proton in strongly acid solution. The ultraviolet spectra of 6-deoxytetracycline (II) and 6-demethyl-6-deoxytetracycline (III) in concentrated sulfuric acid, show a bathochromic shift from 345 to 385 mμ (see Fig. 1). This displacement of the 345 mμ peak must necessarily result from a change in the B, C, D ring chromophore. A reasonable explanation for this shift is the presence of a protonated β-dicarbonyl system in concentrated acid solution. The existence of such a species, IIIa and IIIb, would also explain the inertness of the 11a-carbon atom to an electrophilic halogen (X^+) thus favoring an attack elsewhere in the molecule.



Further confirmation of this ability of the 6-deoxytetracyclines to form the more stabilized protonated β-dicarbonyl system in strong acid solution was obtained by the conversion of 11a-bromo-6-demethyl-6-deoxytetracycline (Va) to 7-bromo-6-demethyl-6-deoxytetracycline (V) in concentrated sulfuric acid solution. In order to demonstrate that this reaction path does not involve an intramolecular rearrangement, the re-

(7) The material, 6-demethyl-6-deoxytetracycline-$7H^3$, was prepared by the method of J. R. D. McCormick, *et al.* (see ref. 5), using 6-demethyltetracyline-$7H^3$ prepared by the method of T. Andre and S. Ullberg, *J. Am. Chem. Soc.*, **79**, 494 (1957).



(8) We wish to thank Dr. E. Ullman for his suggestion of this method of structure determination.

(9) Two methods were used to determine the tritium content in the product; (1) one was to determine the difference in the absolute radioactivity of reactants and products, (2) the second method involved radio scanning of the paper chromatograms of the product. For this latter method a sensitive Radiochromatogram Scanner was loaned to us by Dr. M. Bullock of these laboratories. The technique consisted of slowly passing the paper strip past a counting device which was connected to a recorder that recorded the radioactivity along the paper strip. (b) Treatment of 6-demethyl-6-deoxytetracycline-$7H^3$ with cold sulfuric acid but no N-bromosuccinimide gave back the tetracycline still containing over 80% of the tritium. The small loss was attributed to some tritium–hydrogen exchange under the influence of sulfuric acid.

(10) J. J. Hlavka and D. A. Buyske, *Nature*, **186**, 1064 (1960).

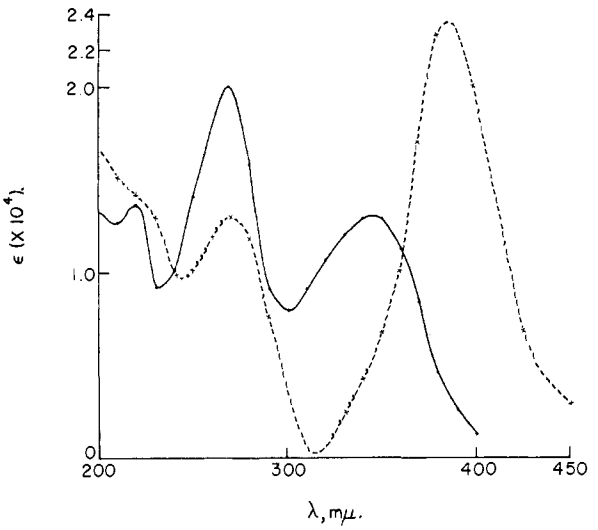
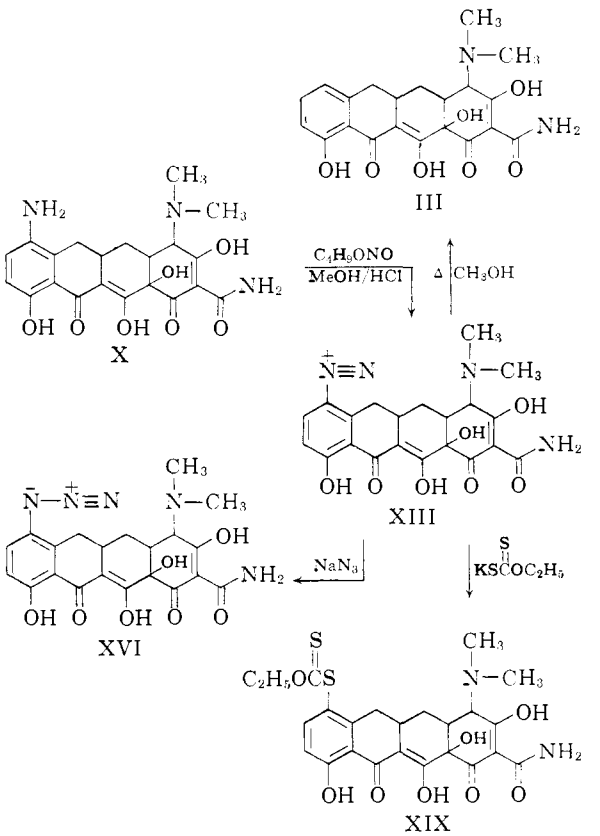


Fig. 1.—Ultraviolet absorption spectra of 6-demethyl-6-deoxytetracycline in 0.1 *N* HCl (———) and concentrated sulfuric acid (- - - -).

action was carried out in the presence of 6-deoxytetracycline (II). In addition to 7-bromo-6-demethyl-6-deoxytetracycline (V) and 6-demethyl-6-deoxytetracycline (III), we isolated the intermolecular brominated product, 7-bromo-6-deoxytetracycline (VII). Moreover, when Va was treated with concentrated sulfuric acid in the presence of α-naphthol, very little (< 10%) brominated tetracycline (V) was obtained, the major product being 6-demethyl-6-deoxytetracycline (III).



Nucleophilic substitution in the D ring was accomplished by first converting the known amino tetracyclines[1] (9-amino-6-deoxytetracycline (IX), 7-amino-6-demethyl-6-deoxytetracycline (X) and 9-amino-6-demethyl-6-deoxytetracycline (XI)) to the corresponding diazonium compounds XII, XIII, XIV. We have found that treatment of any of these amino derivatives with butyl nitrite yields the desired diazonium compound in good yield. In the case of the 9-amino-6-demethyl-6-deoxytetracycline (XI) a mixture (1:2) of diazonium hydrochloride and diazonium oxide was isolated as evidenced by the infrared absorption at 4.45 μ (salt) and 4.65 μ (oxide).

Subsequent displacement of these diazonium groups with either sodium azide or potassium ethylxanthate gave the corresponding azido (XV, XVI, XVII) or ethoxythiocarbonylthio compounds (XVIII, XIX, XX). In contrast to these facile displacements, reaction of 6-deoxy-6-demethyltetracycline-9-diazonium sulfate with boiling methanol yielded only the reduced product, 6-demethyl-6-deoxytetracycline (III).

The relative *in vitro* antibacterial activities of the compounds discussed above are given in Table I.

TABLE I

In Vitro ACTIVITIES IN % COMPARED TO TETRACYCLINE[a]

Tetracycline (I)	100
6-Deoxytetracycline hydrochloride (II)	70
6-Demethyl-6-deoxytetracycline hydrochloride (III)	160
7-Chloro-6-demethyl-6-deoxytetracycline hydrochloride[5] (IV)	300
7-Bromo-6-demethyl-6-deoxytetracycline sulfate (V)	200
7-Iodo-6-demethyl-6-deoxytetracycline sulfate (VI)	120
7-Bromo-6-deoxytetracycline sulfate (VII)	140
7-Iodo-6-deoxytetracycline sulfate (VIII)	60
9-Amino-6-deoxytetracycline hydrochloride (IX)	60
7-Amino-6-demethyl-6-deoxytetracycline hydrochloride (X)	40
9-Amino-6-demethyl-6-deoxytetracycline hydrochloride (XI)	160
6-Deoxytetracycline-9-diazonium disulfate (XII)	10
6-Demethyl-6-deoxytetracycline-7-diazonium sulfate hydrochloride (XIII)	20
6-Demethyl-6-deoxytetracycline-9-diazonium disulfate (XIV)	17
9-Azido-6-deoxytetracycline sulfate (XV)	10
7-Azido-6-demethyl-6-deoxytetracycline sulfate (XVI)	150
9-Azido-6-demethyl-6-deoxytetracycline hydrochloride (XVII)	90
9-Ethoxythiocarbonylthio-6-deoxytetracycline (XVIII)	[b]
7-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline (XIX)	50
9-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline (XX)	10

[a] Activities were measured turbidimetrically against *Staph. aureus* by the method of E. Pelcak and A. Dornbush, *Ann. N. Y. Acad. Sci.*, 51, 218 (1948). [b] This material does not produce the same response curve in the turbidimetric assay; 1/2 maximum inhibition is 0.35γ/ml. as compared to tetracycline of 0.016γ/ml.

The activity data given in Table I allow certain speculations on the structure–activity relationships of these tetracyclines. The introduction of a bromine atom at position 7 increases the ac-

tivity of the parent 6-demethyl-6-deoxytetracyclines (III) twofold. In a similar manner 7-chloro-6-demethyl-6-deoxytetracycline[6] (IV) is three times as active as the non-halogenated compound III. On the other hand, iodine which is less electronegative[11] than either bromine or chlorine has little or no effect on activity. There appears to be a direct relationship between the electronegativity of the halogen in the 7-position and antibiotic activity. This relationship of electronegativity and biological activity may be expanded to include other electron-withdrawing groups.

Experimental[12]

7-Bromo-6-demethyl-6-deoxytetracycline Sulfate[13] (V).—A solution of 0.45 g. (0.1 mmole) of 6-demethyl-6-deoxytetracycline sulfate and 0.2 g. (0.112 mmole) of N-bromosuccinimide in 5.0 ml. of concentrated sulfuric acid was stirred at ice-bath temperature for 30 minutes. The reaction mixture was added dropwise to 250 ml. of cold ether. The solid that separated was filtered and dried; yield 0.51 g. A portion (100 mg.) of this material was recrystallized from a mixture of methanol, chloroform and 2-methoxyethanol; yield 64 mg., $[\alpha]^{25}D$ − 97°, R_f 0.82; $\lambda_{max}^{0.1\ N\ HCl}$ 270, 345 mμ, log ε 4.28, 4.08.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Br \cdot H_2SO_4 \cdot CH_3OH$: C, 42.4; H, 4.3; Br, 13.0; OCH_3, 2.4. Found: C, 42.5; H, 4.7; Br, 13.4; OCH_3, 2.5.

The reaction with 6-demethyl-6-deoxytetracycline-7H[3] was carried out in the same manner.

7-Bromo-6-deoxytetracycline Sulfate[13] (VII).—To a solution of 3.0 g. (6.45 mmoles) of 6-deoxytetracycline hydrochloride in 50.0 ml. of concentrated sulfuric acid at ice-bath temperature was added 1.15 g. (6.45 mmoles) of N-bromosuccinimide. The reaction mixture was stirred at this temperature for 30 minutes, then added dropwise to 4.0 l. of cold ether. The solid that separated was filtered and dried; yield 3.2 g. A portion (100 mg.) of this material was recrystallized twice from methanol–ether; yield 58 mg., $[\alpha]^{25}D$ − 221°, R_f 0.80; $\lambda_{max}^{0.1\ N\ HCl}$ 268, 345 mμ, log ε 4.24, 4.10.

Anal. Calcd. for $C_{22}H_{23}N_2O_7Br \cdot H_2SO_4 \cdot H_2O$: C, 42.3; H, 4.3; N, 4.5; S, 5.1; Br, 12.8. Found: C, 42.3; H, 4.7; N, 4.1; S, 5.3; Br, 13.0.

7-Iodo-6-demethyl-6-deoxytetracycline Sulfate[13] (VI).—To 5.0 ml. of concentrated sulfuric acid cooled in an ice-bath was added 0.512 g. (0.1 mmole) of 6-deoxy-6-demethyltetracycline sulfate followed by 260 mg. (0.115 mmole) of N-iodosuccinimide. The solution was stirred at this temperature for 30 minutes then slowly poured into 300 ml. of ether. The solid was filtered and dried; yield 0.46 g. This material was recrystallized from chloroform–2-methoxyethanol; yield 0.16 g., $[\alpha]^{25}D$ + 383°, R_f 0.91; $\lambda_{max}^{0.1\ N\ HCl}$ 230, 345 mμ, log ε 4.48, 4.12.

Anal. Calcd. for $C_{21}H_{21}N_2O_7I \cdot H_2SO_4 \cdot {}^1/_2H_2O$: C, 39.0; H, 3.7; I, 19.6. Found: C, 39.1; H, 4.2; I, 19.2.

7-Iodo-6-deoxytetracycline Sulfate[13] (VIII).—A solution of 6-deoxytetracycline sulfate (200 mg., 0.38 mmole) and 85 mg. (0.35 mmole) of N-iodosuccinimide in 5.0 ml. of concentrated sulfuric acid was stirred at ice-bath temperature for 40 minutes. The mixture was added dropwise to 250 ml. of cold ether. The solid was filtered and dried; yield 0.16 g. This material was recrystallized from 2-methoxyethanol–chloroform; yield 80 mg., $[\alpha]^{25}D$ − 282°, R_f 0.91; $\lambda_{max}^{0.1\ N\ HCl}$ 240, 260, 345 mμ, log ε 4.26, 4.22, 4.08.

Anal. Calcd. for $C_{22}H_{23}N_2O_7I \cdot H_2SO_4$: N, 4.3; S, 4.9; I, 19.7. Found: N, 3.8; S, 4.8; I, 19.5.

11a-Bromo-6-demethyl-6-deoxytetracycline[14] (Va).—A solution of N-bromosuccinimide (0.21 g., 1.13 mmoles) in glacial acetic acid (8 ml.) was added dropwise with stirring to a solution of 6-demethyl-6-deoxytetracycline hydrochloride (0.50 g., 1.11 mmoles) at 16°. A light yellow solid began to precipitate. After stirring 20 minutes at 16° the solid was filtered from the mixture, washed with ether, and dried in the vacuum desiccator; yield 80%, $[\alpha]^{25}D$ + 33°, R_f 0.64; $\lambda_{max}^{0.1\ N\ HCl}$ 273, 350 mμ, log ε 3.37, 3.48.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Br \cdot HCl$: N, 5.3; Br, 15.1. Found: N, 5.2; Br, 15.0.

Conversion of 11a-Bromo-6-demethyl-6-deoxytetracycline (Va) to 7-Bromo-6-demethyl-6-deoxytetracycline Sulfate (III).—A solution of 11a-bromo-6-demethyl-6-deoxytetracycline (0.10 g., 0.19 mmole) in concentrated sulfuric acid (5 ml.) was stirred at ice-bath temperature for 5 minutes. The reaction mixture was added dropwise to cold ether (250 ml.). The solid that separated was filtered and dried; yield 63 mg. This material was identical in every respect to 7-bromo-6-demethyl-6-deoxytetracycline sulfate.

The addition of α-naphthol to the reaction mixture resulted in the isolation of 6-demethyl-6-deoxytetracycline and less than 10% of the brominated product V.

When the conversion was carried out in the presence of 6-deoxytetracycline, we isolated 7-bromo-6-deoxytetracycline (VIII) and 7-bromo-6-demethyl-6-deoxytetracycline (V).

6-Deoxytetracycline-9-diazonium Disulfate[14] (XII).—To a solution of 9-amino-6-deoxytetracycline disulfate (0.14 g., 0.22 mmole) in 0.1 *N* methanolic hydrochloric acid there was added *n*-butyl nitrite (0.10 ml., 0.9 mmole) with stirring at 0°. The solution was stirred for 30 minutes at 0° and poured into anhydrous ethyl ether (300 ml.). The product was collected by filtration, washed with ether and dried in the vacuum desiccator; yield 70%, $[\alpha]^{25}D$ −169°, R_f 0.0; $\lambda_{max}^{0.1\ N\ HCl}$ 425, 270, log ε 3.55, 4.34.

Anal. Calcd. for $C_{22}H_{22}N_4O_7 \cdot 2H_2SO_4 \cdot 2H_2O$: C, 37.9; H, 4.3; N, 8.2; S, 9.3. Found: C, 37.8; H, 4.7; N, 8.6; S, 9.6.

6-Demethyl-6-deoxytetracycline-7-diazonium Sulfate Hydrochloride[14] (XIII).—To a solution of 1.0 g. (1.9 mmoles) of 7-amino-6-demethyl-6-deoxytetracycline sulfate in 10 ml. of 0.1 *N* hydrochloric acid in methanol at ice-bath temperature was added 1.0 ml. of *n*-butyl nitrite. The solution was stirred at this temperature for 30 minutes and then poured into 500 ml. of cold ether. The solid that separated weighed 0.67 g. This material was not purified any further but was used directly in the displacement reactions. The infrared curve showed strong absorption at 4.45 μ, $[\alpha]^{25}D$ −164°, R_f 0.0; $\lambda_{max}^{0.1\ N\ HCl}$ 255, 310, 345, log ε 4.10, 4.33, 4.74.

6-Demethyl-6-deoxytetracycline-9-diazonium Disulfate[14] (XIV).—A solution of 0.5 g. (0.80 mmole) of 9-amino-6-demethyl-6-deoxytetracycline disulfate in 10 ml. of 0.1 *N* methanolic hydrochloric acid (8.3 ml. of concentrated hydrochloric acid in 1 l. of methanol) was cooled to 0°, and 0.5 ml. (4.4 mmoles) of *n*-butyl nitrite was added. After being stirred at 0–5° for 30 minutes, the reaction solution was slowly poured into 150 ml. of cold, anhydrous ethyl ether. The product which precipitated was collected by filtration, washed, and dried to give 400 mg. of a hygroscopic, yellow solid, $[\alpha]^{25}D$ − 23°, R_f 0.0; $\lambda_{max}^{0.1\ N\ HCl}$ 270, 320, 430 mμ, log ε 4.38, 3.87, 3.98.

Anal. Calcd. for $C_{21}H_{21}N_4O_7 \cdot 2H_2SO_4 \cdot H_2O$: C, 38.4; H, 4.1; N, 8.5. Found: C, 38.53; H, 4.24; N, 8.71.

9-Azido-6-deoxytetracycline Sulfate[14] (XV).—To a solution of 6-deoxytetracycline-9-diazonium disulfate (0.20 g., 0.30 mmole) in 0.1 *N* methanolic hydrochloric acid (10 ml.) there was added sodium azide (0.022 g., 0.33 mmole) with stirring at 30°. After stirring for 1.5 hours the solution was poured into anhydrous ethyl ether and the precipitate was collected by filtration.

The crude product was dissolved in methanol at room temperature and brought to the cloud point with ether and filtered. This process was repeated three times on the subsequent filtrates in order to obtain the product in crystalline form; yield 30%, $[\alpha]^{25}D$ − 220°; $\lambda_{max}^{0.1\ N\ HCl}$ 345, 250, log ε 4.03, 4.28.

Anal. Calcd. for $C_{22}H_{23}N_5O_7 \cdot H_2SO_4$: N, 12.3. Found: N, 12.2.

7-Azido-6-demethyl-6-deoxytetracycline Sulfate[14] (XVI).—A solution of 3.0 g. (4.65 mmoles) of 6-demethyl-6-deoxytetracycline-7-diazonium sulfate hydrochloride and 0.33 g.

(11) L. Pauling, "The Nature of the Chemical Bond," 2nd ed., Cornell University Press, Ithaca, N. Y., 1945, pp. 58–75.

(12) R_f values were determined in the system 1-butanol–0.2 *M* phosphate buffer, *p*H 2.

(13) Optical rotation was determined at a concentration of 0.1–0.5% in 0.1 *N* sulfuric acid.

(14) Optical rotation was determined at a concentration of 0.1–0.5% in 2-methoxyethanol.

(5.25 mmole) of sodium azide in 90 ml. of 0.1 *N* hydrochloric acid in methanol was stirred at room temperature for 2 hours. The solution was poured into 2.0 l. of cold ether and the solid that separated was filtered and dried; yield 2.5 g. This material was recrystallized from methanol–ether; yield 1.25 g., $[\alpha]^{25}D$ −185°, R_f 0.77; $\lambda_{max}^{0.1\ N\ HCl}$ 255, 340, log ε 4.33, 3.92.

Anal. Calcd. for $C_{21}H_{22}N_6O_7 \cdot H_2SO_4 \cdot H_2O$: C, 44.1; H, 4.6; N, 12.2; S, 5.6. Found: C, 43.9; H, 4.7; N, 11.9; S, 5.6.

9-Azido-6-demethyl-6-deoxytetracycline Hydrochloride[14] (**XVII**).—Sodium azide (0.75 g., 11.5 mmoles) was added to 5.0 g. (10.4 mmoles) of 6-demethyl-6-deoxytetracycline-9-diazonium oxide hydrochloride in 125 ml. of 0.1 *N* methanolic hydrochloric acid (8.3 ml. of concentrated hydrochloric acid in 1 liter of methanol). The solution was stirred at room temperature for 45 minutes, and the solid which precipitated was filtered and dried to give 2.5 g. The crude product was recrystallized from methanol–ethyl ether; 1.5 g. of a yellow, crystalline solid was obtained, $[\alpha]^{25}D$ − 230°, R_f 0.74; $\lambda_{max}^{0.1\ N\ HCl}$ 260, 345 mμ, log ε 4.30, 4.10.

Anal. Calcd. for $C_{21}H_{21}N_5O_7 \cdot HCl \cdot H_2O$: C, 49.6; H, 4.7; N, 13.7; Cl, 7.0. Found: C, 50.07; H, 4.21; N, 13.35; Cl, 7.33.

9-Ethoxythiocarbonylthio-6-deoxytetracycline[14] (**XVIII**).—A solution of 6-deoxytetracycline-9-diazonium disulfate (1.0 g., 1.5 mmoles) in water (20 ml.) was added dropwise with stirring to a solution of potassium ethyl xanthate (1.7 g., 11 mmoles) in water (20 ml.). The mixture was stirred for 1 hour and the solid which precipitated out of solution was collected and dried in the vacuum desiccator; yield 70%.

The product was purified by partition column chromatography (solvent system: ethyl acetate, 60; heptane, 40; 0.2 *M* phosphate buffer, *p*H 2.0, 20), $[\alpha]^{25}D$ − 174°, R_f 0.81; $\lambda_{max}^{0.1\ N\ HCl}$ 255, 275, 350, log ε 3.23, 3.23, 3.10.

Anal. Calcd. for $C_{25}H_{28}N_2O_8S_2$: N, 5.1; S, 11.7. Found: N, 4.7; S, 12.1.

7-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline[14] (**XIX**).—A solution of 2.0 g. (3.4 mmoles) of 6-demethyl-6-deoxytetracycline-7-diazonium hydrochloride sulfate (XIII) and 0.54 g. (3.4 mmoles) of potassium ethyl xanthate in 30 ml. of water was stored at room temperature until the evolution of nitrogen ceased (10 min.) and the solution was freeze-dried; yield 2.0 g. This material was purified by partition chromatography using the solvent system chloroform–butanol–*p*H 2 buffer (200:5:100) and diatomaceous earth as the support for the stationary phase; $[\alpha]^{25}D$ − 96.5°, R_f 0.77; $\lambda_{max}^{0.1\ N\ HCl}$ 245, 270, 355, log ε 4.39, 4.25, 4.10.

Anal. Calcd. for $C_{24}H_{26}N_2O_8S_2 \cdot H_2O$: C, 52.2; H, 5.4; N, 4.7; S, 11.6. Found: C, 52.2; H, 5.1; N, 5.0; S, 11.0.

9-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline[14] (**XX**).—A solution of 1.5 g. (9.4 mmoles) of potassium ethyl xanthate in 25 ml. of water was slowly poured into 1.9 g. (3.6 mmoles) of 6-methyl-6-deoxytetracycline-9-diazonium oxide hydrochloride in 40 ml. (4.0 mmoles) of 0.1 *N* hydrochloric acid. After 45 minutes of stirring at room temperature, the *p*H of the solution was adjusted from 3.2 to 4.5 with 1 *N* sodium hydroxide to precipitate the free base. Filtering off the precipitate and drying gave a 1.2 g. yield, $[\alpha]^{25}D$ − 51°, R_f 0.86; $\lambda_{max}^{0.1\ N\ HCl}$ 265, 345 mμ, log ε 4.34, 4.10.

Anal. Calcd. for $C_{24}H_{26}N_2O_8S_2$: N, 5.3; S, 12.0. Found: N, 5.23; S, 12.47.

Reduction of 6-Demethyl-6-deoxytetracycline-9-diazonium Disulfate (XIII) to 6-Dimethyl-6-deoxytetracycline (III).—A solution of 100 mg. of XIII in 10 ml. of methanol was heated to reflux for 10 minutes and evaporated to dryness *in vacuo*. The residue was identical to 6-demethyl-6-deoxytetracycline as compared by ultraviolet and infrared spectroscopy and paper chromatography.

Reduction of 6-Deoxytetracycline-9-diazonium Disulfate (XII) to 6-Deoxytetracycline Sulfate (II).—A solution of 6-deoxytetracycline-9-diazonium disulfate (0.10 g., 0.55 mmole) in methanol (10 ml.) was refluxed under anhydrous conditions for 30 minutes. The reaction mixture was added dropwise to 400 ml. of ethyl ether and the solid which separated was filtered and dried. This material was identical to 6-deoxytetracycline sulfate as shown by paper chromatography and infrared and ultraviolet spectroscopy.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIFORNIA]

Mass Spectrometry in Structural and Stereochemical Problems. I. Steroid Ketones[1]

BY HERBERT BUDZIKIEWICZ AND CARL DJERASSI

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Mass spectra have been measured for steroids with keto groups in all possible eleven nuclear positions. Characteristic features have been noted (largely due to primary cleavage of the carbon–carbon bond in the ring adjacent to the carbonyl group and retention of the charge with the oxygen-containing fragment) which in most instances permit a decision as to the location of the carbonyl group in a steroid skeleton. It should now be possible to locate in an unambiguous manner a carbonyl group in a saturated, monoketonic steroid by the combined use of mass spectrometry and optical rotatory dispersion.

In spite of the extensive applications of mass spectrometry[2] to a wide variety of organic compounds, only very few systematic studies have been performed with steroids. Reed[3] has concentrated on the use of low-voltage spectra for the determination of the molecular weight and the size of the side chain in sterol and triterpene types, but he has also listed the most important fragments in the high voltage spectra of a few members of these classes. Friedland and collaborators[4] discussed the principal fragmentation processes in a rather heterogeneous group of steroid alcohols, while Bergström and his colleagues[5] emphasized the use of mass spectrometry for the determination of the molecular weights of bile acids. A more detailed discussion of the fragmentation patterns observed with such bile acids has been published recently by Ryhage and Stenhagen,[6] who have also carried out a very extensive mass spectro-

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(2) See J. H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier Publ. Co., Amsterdam, 1960.

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