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Some Transformations at the 12a-Position in the Tetracycline Series[1]

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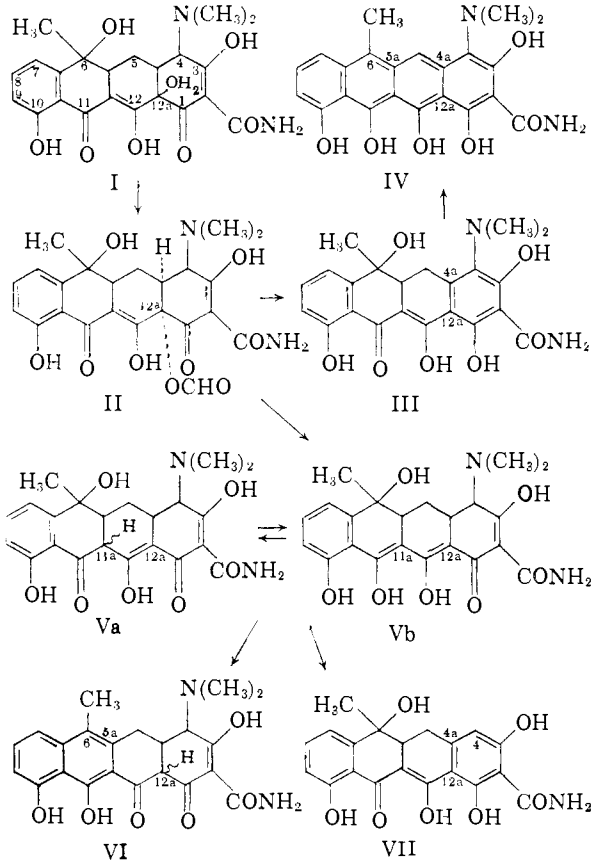
O^{12a}-Formyltetracycline (II), an ester with interesting chemical and antimicrobial properties, has been prepared. Structural assignment of II rests on novel types of tetracycline degradation. These are: (1) the facile conversion of II to 4a,12a-anhydrotetracycline (III), and thence to terrarubein (IV, 4a,12a;5a,6-dianhydrotetracycline), a previously reported degradation product of 5-hydroxytetracycline; and (2) the catalytic hydrogenolysis of II to 12a-deoxytetracycline (V). Similar transformations have been carried out on other O^{12a}-acylated tetracyclines and furnish an additional tool for the structural assignment of such derivatives.

In the course of acylation studies[2] with tetracycline[3](I), a *monoformate, O^{12a}-formyltetracycline* (II), has been prepared. This ester is of chemical interest because it represents an entree to novel degradation reactions involving the 12a-position. Compound II is also of biological interest due to its antimicrobial properties.[1,4]

O^{12a}-Formyltetracycline (II) is formed by treating a cold pyridine solution of tetracycline (I) with excess acetoformic acid reagent.[5] The presence of a formate ester group was first deduced by the observation of a strong infrared peak at 5.84 μ and verified by elemental analyses and a formyl determination. These observations, together with the transformations described below, leave no doubt as to the structure, II, of the ester.[6]



O^{12a}-Formyltetracycline rapidly undergoes solvolysis to tetracycline in basic or in neutral solution. For example, a sample of the formate ester dissolved in warm methanol and allowed to stand a few minutes before acidification shows the ultraviolet absorption of tetracycline. In contrast, a sample dissolved in cool, preacidified methanol shows the ultraviolet absorption of the formate.[7] By following the change with time of the ultraviolet absorption of dilute solutions of O^{12a}-formyltetracycline in aqueous buffers (selected so as to be free of complexing agents), approximate half-lives for the hydrolysis to tetracycline at 25° were shown to be

*p*H	2.0	4.0	6.0	7.5
$t_{1/2}$, hr.	6	4	0.5	5 min.

One of the new reactions which provides a proof of structure for O^{12a}-formyltetracycline was quite unexpected. Since the formate was first isolated as a hydrate, an attempt was made to obtain the anhydrous material *via* azeotropic removal of water with boiling toluene. We were surprised to find that complete degradation of the formate occurs in this process. The formyl group is lost as formic acid and a red crystalline product analyzing for an anhydrotetracycline is isolated in high yield. It is apparent from spectral data that this product is not the well known 5a,6-anhydrotetracycline[8] (derived from a possible formoxy group at the 6-position) or tetracyclinonitrile[2] (derived from a possible formyl group on the amide oxygen). The most reasonable interpretation is that the ester has a formoxy group at the 12a-position and

(1) Preliminary communication, R. K. Blackwood, H. H. Rennhard and C. R. Stephens, THIS JOURNAL, **82**, 745 (1960).

(2) C. R. Stephens and co-workers, unpublished work, Chas. Pfizer and Co., Inc.

(3) Tetracyn and Achromycin are the registered trade marks of Chas. Pfizer and Co., Inc., and American Cyanamid Co., respectively, for the antibiotic tetracycline.

(4) We are indebted to Drs. A. R. English and T. J. McBride for antimicrobial investigations.

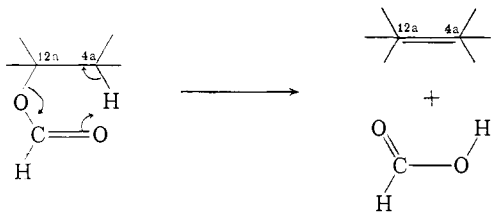
(5) A solution of acetic–formic anhydride in acetic acid; *cf.* V. C. Mehlenbacher in "Organic Analyses," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 37.

(6) Prior to the observed 12a-transformations, formylation of the amide group of tetracycline was considered to be a distinct possibility. Consistent with this early view was the isolation of *N-formylsalicylamide* under the same conditions employed for the preparation of II. Although a similar parallelism has been used in support of structural assignment for other tetracycline derivatives, *viz.*, the aminomethyltetracyclines, [W. J. Gottstein, W. F. Minor and L. C. Cheney, THIS JOURNAL, **81**, 1198 (1959)] it is manifest that such a comparison must be used with caution.

(7) This absorption differs slightly (350 mμ region) from that of tetracycline. Confirmation that these observations indeed reflect hydrolysis was obtained by paper chromatographic studies and by isolation of tetracycline.

(8) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952); L. H. Conover, W. T. Moreland, A. R. English, C. R Stephens and F. J. Pilgrim, *ibid.*, **75**, 4622 (1953).

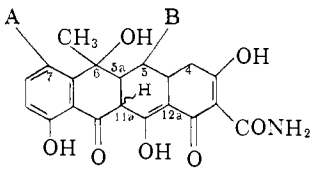
that the new product is *4a,12a-anhydrotetracycline* (III), formed by pyrolytic *cis*-elimination[9,10] of formic acid



Confirmation of this interpretation is seen in the further conversion of III, by mild acid treatment into 5a,6;4a,12a-dianhydrotetracycline (IV, terrarubein), a previously reported degradation product of 5-hydroxytetracycline.[11,12] Compound IV represents the only common 5-hydroxytetracycline degradation product reported to date.

4a,12a-Anhydrotetracycline retains a modicum of the *in vitro* antibacterial activity of tetracycline.[4] The substance is tautomeric in nature, as reflected by its ultraviolet absorption behavior in dilute, acid–methanol solution.[13] Thus the intensities of the various maxima change until such time as tautomeric equilibrium is established (*ca.* 30–45 minutes). A marked drop in the intensity of twin peaks at 405–425 mμ during this process is particularly striking.

A second structurally definitive, novel reaction of the formate II is its catalytic hydrogenolysis, in good yield, to 12a-deoxytetracycline(V).[14] The structure of this derivative was established by its elemental analyses, by the degradations described below, and by comparison of its ultraviolet absorption to those of two previously reported compounds: 12a-deoxy-4-dedimethylamino-5-hydroxytetracycline (IX)[11] and 12a-deoxy-4-dedimethylamino-7-chlorotetracycline (X).[15]

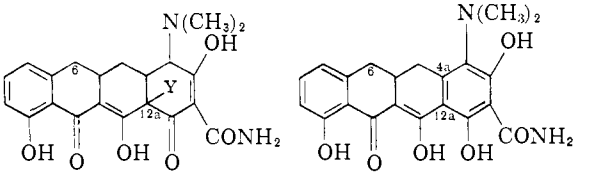


IX, A = H, B = OH
X, A = Cl, B = H

In contrast to IX and X, 12a-deoxytetracycline shows[13] not only an ultraviolet chromophore (323 mμ) accounted for by a tautomer with a proton at the 11a-position[11] (such as Va, IX and X), but an additional, much longer wave length chromophore (429 and 450 mμ) which is undoubtedly represented by a more highly conjugated form such as Vb.[16] In acid–methanol[13] the tautomers achieve equilibrium after about 2 hours, at which time the 323 mμ absorbing species predominates.

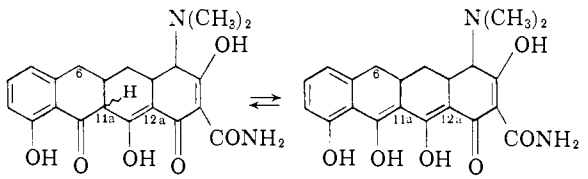
12a-Deoxytetracycline (V) retains an appreciable amount of the antibacterial activity of tetracycline.[4] Reoxidation of V to tetracycline has been reported.[17] Under mild acid conditions, V is degraded to 5a,6-anhydro-12a-deoxytetracycline (VI), a compound identified by the close similarity of its ultraviolet absorption to that of the corresponding 5a,6-anhydro product[15] derived from X. Treatment of V with methyl iodide and propylene oxide in refluxing tetrahydrofuran produces tetramethylammonium iodide and *4-dedimethylamino-4a,12a-anhydrotetracycline* (VII). The latter was also characterized by its ultraviolet absorption,[13] which bears a detailed resemblance to that of 4a,12a-anhydrotetracycline (III).

Transformations similar to those described above have also been carried out with 6-demethyl-6-deoxytetracycline (XI).[18] Thus treatment of XI (hydrochloride salt) in pyridine with acetoformic acid reagent[5] in the cold produces a *crude O*^{12a}*-formyl-6-demethyl-6-deoxytetracycline* (XII). Refluxing XII in toluene yields **4a,12a-anhydro-6-demethyl-6-deoxytetracycline (XIII)**, while catalytic hydrogenolysis of XII yields *6,12a-dideoxy-6-*



XI, Y = OH
XII, Y = OCHO

XIII



XIV

(9) *Cf.* L. H. Conover, "Special Publication No. 5," The Chemical Society, London, 1956, p. 77 *et seq.*, for a discussion of the *cis*-AB ring junction ascribed to the tetracycline antibiotics.

(10) *Cf.* D. J. Cram in "Steric Effects in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 305 *et seq.*, for a discussion of *cis* elimination reactions.

(11) 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, THIS JOURNAL, **75**, 5455 (1953). The present process represents a superior method for the preparation of IV.

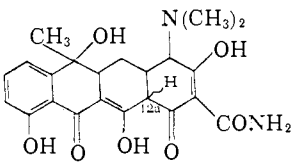
(12) Terramycin is the registered trade mark of Chas. Pfizer and Co., Inc., for the antibiotic 5-hydroxytetracycline. The generic name of this substance is oxytetracycline.

(13) Methanol, 10^{-2} *N* in hydrochloric acid, *ca.* 2×10^{-5} *M* (1 mg./100 ml.) in the derivative under study.

(14) The independent preparation of 12a-deoxytetracycline, by zinc–ammonium hydroxide reduction of tetracycline, has been reported in Belgian Patent 572,382 and by A. Green and J. H. Booth, THIS JOURNAL, **82**, 3950 (1960).

(15) 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).

(16) An enolic form with a proton at 12a (Vc) is not considered to make an important contribution. Otherwise, the ultraviolet absorption should be much more similar to the tetracyclines themselves or to 12a-*epi*-4-dedimethylamino-5-hydroxytetracycline (*cf.* refs. 9, 11 and 15).



Vc

(17) C. E. Holmlund, W. W. Andres and A. J. Shay, THIS JOURNAL, **81**, 4748, 4750 (1959).

(18) C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover and K. J. Brunings, *ibid.*, **80**, 5324 (1958).

demethyltetracycline (XIV). These derivatives (XIII and XIV) show spectral properties closely analogous to those of the corresponding tetracycline derivatives III and V.

In contrast to tetracycline and 6-demethyl-6-deoxytetracycline, 5-hydroxytetracycline undergoes a more complex reaction with acetoformic acid reagent[5] in pyridine. A *diformate* of incompletely determined structure is formed. This product undergoes degradative reactions of a different type.[2]

Finally, the reactions described herein do not appear to be unique for formates. Thus *O^{12a}-phenylcarbamyltetracycline*[2] is indicated, by ultraviolet spectra on crude products, to be converted to 12a-deoxytetracycline (*via* catalytic reduction) and to 4a,12a-anhydrotetracycline (*via* an elimination reaction in refluxing toluene). Similarly, *O^{10},O^{12a}-diacetyl-5-hydroxytetracycline*[2] *O^{10},O^{12a}-dipropionyl-5-hydroxytetracycline*[2] *and crude O^{12a}-phenylcarbamyl-5-hydroxytetracycline*[2] are hydrogenolyzed to crude 12a-deoxy derivatives. These transformations offer an additional tool useful for the assignment of structure to acylated derivatives in the tetracycline series.

Experimental

O^{12a}-Formyltetracycline (II).—Anhydrous, amphoteric tetracycline (I, 10 g., recrystallized from toluene) was dissolved by stirring in 20 ml. of pyridine cooled in an ice–water-bath. Acetoformic acid reagent[5] (20 ml.) was added dropwise to the stirred solution at a rate which maintained the temperature at less than 10° (*ca.* 15 minutes). After an additional 15 minutes in the bath, the reaction mixture was poured into 60 ml. of ice and water. The *p*H was immediately adjusted to *ca.* 3.5, whereupon II crystallized as the monohydrate. The product was isolated, washed with water, then acetone, and air-dried. The yield of II (hydrate) was 6.8 g. (61%).

Anal. Calcd. for $C_{23}H_{24}O_9N_2 \cdot H_2O$: C, 56.3; H, 5.3; N, 5.7; CHO, 5.8; H_2O, 3.7. Found: C, 56.5; H, 5.3; N, 5.7; CHO, 4.6; H_2O, 4.7. Calcd. for $C_{23}H_{24}O_9N_2$ (sample dried *in vacuo* at 100°): C, 58.5; H, 5.1; N, 5.9. Found: C, 58.5; H, 5.3; N, 6.1.

Alternatively, II may be isolated in almost quantitative yield as a crude, amorphous product suitable for further transformations. Instead of ice and water, the pyridine reaction mixture described above was added dropwise to 300 ml. of stirring diethyl ether. The precipitated product was recovered by filtration through a medium porosity, sintered glass funnel with thorough reslurries and washes with ether.

The hydrochloride of II was prepared by the following process: The hydrate of II (25 g.) was slurried in 200 ml. of cool methanol. A solution of 7.5 ml. of concd. hydrochloric acid in 50 ml. of methanol was added. The solution was filtered immediately into 2.5 liters of ether. The precipitate so formed was recovered by filtration and washed well with ether. The amorphous product was crystallized by stirring with 250 ml. of acetone for 2 hours. The yield of the hydrochloride of II, dried *in vacuo* at 60–65°, was 18.7 g.

Anal. Calcd. for $C_{23}H_{24}O_9N_2 \cdot HCl$: C, 54.3; H, 5.0; N, 5.5; Cl^-, 7.0. Found: C, 54.1; H, 5.2; N, 5.5; Cl^-, 6.8.

All forms of II described above show a strong infrared peak in the range 5.76 to 5.84 μ (KBr pellet). When spotted in a variety of suitable papergram systems, they show, if dissolved in a solvent such as acetone which will not lead to solvolysis prior to application to the paper, a higher R_f spot together with a streak to the R_f of tetracycline. When either the crystalline amphoteric form or the hydrochloride is dissolved in preacidified methanol,[13] the following ultraviolet spectrum is recorded: λ_{max} 267 mμ, log ϵ 4.29, and λ_{max} 360 mμ, log ϵ 4.07. A sample of hydrated II dissolved in methanol (warming is necessary for any but very dilute solutions such as 1 mg./100 ml.) shows the ultraviolet absorption[13] and paper chromatographic behavior of tetracycline. A sample so dissolved and then evaporated to a dry solid no longer shows an ester band in the infrared and is (on crystallization by brief stirring in water) identical by all criteria to hydrated tetracycline.

The aqueous media used to determine the approximate half-lives for the hydrolysis of II (hydrochloride) to tetracycline were: *p*H 2, 0.01 *N* hydrochloric acid; *p*H 4, 0.025 *M* acetic acid adjusted with tetramethylammonium hydroxide; *p*H 6, 0.0125 *M* acetic acid and 0.0125 *M* phosphoric acid adjusted with tetramethylammonium hydroxide; and *p*H 7.5, 0.025 *M* phosphoric acid adjusted with tetramethylammonium hydroxide. Half-lives were obtained by selecting a wave length (295, 310, 265 and 312 mμ for *p*H's 2, 4, 6 and 7.5, respectively) at which the over-all change in ultraviolet absorption from II to I was greatest and taking $t_{1/2}$ as the time when half of the over-all intensity change had occurred. Solutions were kept at 25° in a water-bath.

4a,12a-Anhydrotetracycline (III).—Freshly prepared amorphous, anhydrous O^{12a}-formyltetracycline (23.5 g. derived from 22.5 g. of tetracycline) was suspended in 980 ml. of toluene. The mixture was refluxed gently for 20 hours, with collection of formic acid in a Dean–Stark trap. The mixture was cooled, filtered and stripped to yield crude III. The crude was taken up in 600 ml. of hot ethylene dichloride, filtered and reduced in volume to *ca.* 100 ml. to produce 9.8 g. of pure III (43% yield, over-all from tetracycline). Further crops from the mother liquor, while indicating on assay a very high conversion to III, were increasingly contaminated with tetracycline and *epi*-tetracycline.

Compound III was earlier prepared from hydrated II by the same process. An ultraviolet assay (employing the 405–425 mμ absorption bands) indicated *ca.* 80% conversion to III, a figure in agreement with the 70% yield of formic acid collected in the Dean–Stark trap (determined by titration, identified by its pK_a and by conversion to its *p*-bromophenacyl ester). Product III was isolated, crystallized as above, and dried at 100° *in vacuo*. The ultraviolet spectrum,[13] after standing 30–40 minutes to achieve tautomeric equilibrium, shows λ_{max} 247 mμ, log ϵ 4.29; λ_{max} 329 mμ, log ϵ 3.82; λ_{max} 405 mμ, log ϵ 4.33; λ_{max} 426 mμ, log ϵ 4.35. This spectrum and the elemental analyses were not changed by recrystallization from toluene or from ethanol–water.

Anal. Calcd. for $C_{22}H_{22}N_2O_7$: C, 62.0; H, 5.2; N, 6.6. Found: C, 61.8, 62.0, 62.0; H, 5.3, 5.4, 5.3; N, 6.5, 6.3, 6.4.

5a,6;4a,12a-Dianhydrotetracycline (Terrarubein, IV).—4a,12a-Anhydrotetracycline (0.1 g.) was dissolved in 4 ml. of acetone with stirring. Concentrated hydrochloric acid (0.3 ml.) was added, and the mixture was warmed to 50° for 6 minutes, then cooled and filtered to yield 0.1 g. of IV (dried at 100° *in vacuo*). This product showed an infrared spectrum identical to IV prepared earlier from 5-hydroxytetracycline.[11]

Anal. Calcd. for $C_{22}H_{20}N_2O_6$: C, 64.7; H, 4.9; N, 6.8; Cl^-, 0.0. Found: C, 64.5; H, 4.9; N, 6.8; Cl^-, 0.0.

12a-Deoxytetracycline (V).—O^{12a}-Formyltetracycline (48.2 g. of amorphous, anhydrous material from 50 g. of tetracycline, extensively washed with ether) was dissolved in 500 ml. of tetrahydrofuran. Palladium (5%)-on-carbon (20 g.) was added, and the mixture was hydrogenated in a 1-liter stirred autoclave at 55 ± 2° and 100 p.s.i. for 23 hours. The catalyst was filtered and washed with tetrahydrofuran. After the addition of 500 ml. of methanol, the filtrate was concentrated *in vacuo*. During this process, pure V, as a methanol solvate, formed as fine brick-red crystals; yield 21.7 g. (42% over-all from tetracycline). The ultraviolet spectrum[13] (immediate) shows λ_{max} 264 mμ, log ϵ 4.25; λ_{max} 325 mμ, log ϵ 4.12; λ_{max} 429 mμ, log ϵ 4.05; λ_{max} 450 mμ, log ϵ 3.98. After standing *ca.* 2 hours to achieve tautomeric equilibrium, it shows λ_{max} 265 mμ, log ϵ 4.34; λ_{max} 323 mμ, log ϵ 4.26; λ_{max} 430 mμ, log ϵ 3.59.

Anal. Calcd. for $C_{22}H_{24}N_2O_7 \cdot CH_3OH$: C, 60.0; H, 6.1; N, 6.1; OCH_3, 6.7. Found: C, 60.2; H, 6.0; N, 5.9; OCH_3, 6.5.

(19) We are indebted to Mr. T. J. Toolan and his associates for physical measurements and to Messrs. J. A. Aimetti, R. A. Bliss, L. U. Broom and B. P. Turgeon for technical assistance.

5a,6-Anhydro-12a-deoxytetracycline (VI).—A suspension of 6 g. of 12a-deoxytetracycline methanolate in 30 ml. of 2 *N* methanolic hydrochloric acid was heated under reflux for 1 hour. A transient yellow precipitate was transformed by this process into a heavy red crystalline precipitate. This was filtered, washed with methanol and ether, and dried. The yield of VI (HCl) was 5.6 g. Its ultraviolet spectrum[13] shows λ_{max} 272 mμ, log ε 4.52; λ_{max} 325 mμ, log ε 3.95; λ_{max} 378 mμ, log ε 4.12; λ_{max} 434 mμ, log ε 4.34.

Anal. Calcd. for $C_{22}H_{22}N_2O_6 \cdot HCl$: C, 59.3; H, 5.2; N, 6.3. Found: C, 59.5; H, 5.4; N, 6.4.

4a,12a-Anhydro-4-dedimethylaminotetracycline (VII).—A mixture of 2 g. of 12a-deoxytetracycline, 175 ml. of tetrahydrofuran (distilled from sodium borohydride), 10 ml. of methyl iodide and 10 ml. of propylene oxide was heated under reflux for 24 hours. After overnight refrigeration, filtration of the cold mixture gave 1.5 g. of crude VII. The mother liquor was concentrated to half-volume, treated with an additional 5 ml. each of methyl iodide and propylene oxide and then heated for an additional 24 hours to yield another 0.5 g. of crude VII. The crudes were combined, stirred in 10 ml. of warm water (60°) for 20 minutes to extract tetramethylammonium iodide, filtered, washed with water and dried to yield 1.2 g. of partially purified VII. The latter was dissolved in 100 ml. of hot dimethylformamide, treated with Darco G-60, and the product caused to crystallize by the addition of methanol. The yield of VII was 760 mg. (46%). Compound VII shows an ultraviolet spectrum[13] with λ_{max} 247, 404 and 424 mμ.

Anal. Calcd. for $C_{20}H_{17}NO_7$: C, 62.7; H, 4.5; N, 3.7. Found: C, 62.7; H, 4.6; N, 3.8.

Crude O^{12a}-Formyl-6-demethyl-6-deoxytetracycline (XII).—6-Demethyl-6-deoxytetracycline hydrochloride[18] (XI, 10 g.) was dissolved in 20 ml. of pyridine and cooled to <5° in an ice-bath. Acetoformic acid reagent[5] (15 ml.) was added dropwise over 10 minutes so as to keep the internal temperature *ca.* 10–12°. After stirring a further 10 minutes, the reaction mixture was poured slowly into 300 ml. of rapidly stirring anhydrous ether. Crude XII, 12.8 g., was recovered by filtration on a medium porosity, sintered glass funnel with repeated reslurries in ether. The product was stored in a vacuum desiccator until its use within a day or two. The infrared spectrum on this solid is similar to that of the corresponding amorphous anhydrous formate II from tetracycline.

4a,12a-Anhydro-6-demethyl-6-deoxytetracycline (XIII).—Freshly prepared crude formate (XII, 12.7 g.) was heated under reflux for 40 hours in 125 ml. of toluene, with collection of formic acid in a Dean–Stark trap. The hot mixture was filtered. The filtrate, on stripping to dryness, gave 7.0 g. of crude XIII (*ca.* 90% by ultraviolet assay). Recrystallization from ethylene dichloride gave 4.3 g. of pure XIII (49% yield over-all from XI). The ultraviolet spectrum[13] shows (after shaking *ca.* 45 minutes to dissolve and achieve tautomeric equilibrium) λ_{max} 248 mμ, log ε 4.24; λ_{max} 330 mμ, log ε 3.69; λ_{max} 403 mμ, log ε 4.48; λ_{max} 423 mμ, log ε 4.49.

Anal. Calcd. for $C_{21}H_{20}N_2O_6$: C, 63.6; H, 5.1; N, 7.1. Found: C, 63.6; H, 5.1; N, 7.1.

6-Demethyl-6,12a-dideoxytetracycline (XIV).—Crude XII (1 g.) was dissolved in 5 ml. of dimethylformamide and, following addition of 0.5 g. of 5% palladium-on-carbon, hydrogenated for 7 hours at room temperature and 1 atmosphere pressure. The catalyst was removed by filtration, with 5 ml. of dimethylformamide used for transfer and washing. On addition of an equal volume of water to the filtrate, XIV crystallized (70 mg., 10% over-all yield from XI). The ultraviolet spectrum[13] (after standing overnight to achieve tautomeric equilibrium) shows λ_{max} 266 mμ, log ε 4.28; λ_{max} 323 mμ, log ε 4.24; λ_{max} 427 mμ, log ε 3.83; λ_{max} 439 mμ, log ε 3.75.

Anal. Calcd. for $C_{21}H_{22}N_2O_6$: C, 63.3; H, 5.6; N, 7.0. Found: C, 62.7; H, 5.6; N, 6.9.

N-Formylsalicylamide.—Salicylamide (10 g.) was stirred with 60 ml. of pyridine and 45 ml. of acetoformic acid reagent[5] for 1 hour at 0–3° and 1.5 hours at room temperature. The reaction mixture was poured into 50 ml. of ice and water. The aqueous solution was extracted with six 50-ml. portions of ether. The combined extracts were dried over magnesium sulfate, filtered and stripped to dryness on a rotating evaporator. Methylcyclohexane was added to the resulting oil and the mixture re-evaporated to yield a dry solid. Paper chromatography (4 systems) indicated the crude to be a mixture of salicylamide and N-formylsalicylamide (identified below). Recrystallizations from benzene, acetone and finally chloroform gave pure N-formylsalicylamide (m.p. 161–162°). This product, showing only a single component in four different paper chromatography systems, was identified by analysis and by the close analogy of its ultraviolet absorption in methanol–0.01 *N* HCl (λ_{max} 247 mμ, log ε 4.12; λ_{max} 311 mμ, log ε 3.63) and methanol–0.01 *N* NaOH[20] (λ_{max} 259 mμ, log ε 3.95; λ_{max} 360 mμ, log ε 3.81) to that of commercially available N-acetylsalicylamide (λ_{max} 242 and 306 mμ in acid, 255 and 351 mμ in base, with relative peak heights almost identical to those of the formate). At the same time, the ultraviolet absorption of N-formylsalicylamide (λ_{min} 290 mμ, log ε 2.54, in methanol–0.01 *N* NaOH[20]) is very much different from that of commercially available O-acetylsalicylamide which shows nearly the same ultraviolet absorption in both acid and base (λ_{max} 288–290 mμ).

Anal. Calcd. for $C_8H_7NO_3$: C, 58.2; H, 4.3; N, 8.5. Found: C, 58.3; H, 4.3; N, 8.5.

(20) Run immediately after dissolution, since hydrolysis to salicylamide is observed under these conditions.