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Bromination of Tetracycline*

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Tetracycline (I) reacts with bromine in the presence of pyridine to form 11*a*-bromotetracycline-6,12-hemiketal (II). Dehydration of II, preferably in a mixture of sulfuric acid and acetonitrile, yields 11*a*-bromo-6-methylenetetracycline (III) as sulfate, which affords 6-methylenetetracycline (VI) upon reduction. Evidence to support the structures of these products comes from elementary analysis, UV, IR and NMR data.

It is well known[1–4] that amphoteric tetracycline (I) reacts with *N*-chlorosuccinimide or perchlorylfluoride to form 11*a*-chlorotetracycline-6,12-hemiketal and 11*a*-fluorotetracycline-6,12-hemiketal respectively. These substances are the key intermediates in the conversion of compound I to the fully biological active 6-methylenetetracycline (VI), and they undergo exocyclic dehydration in liquid hydrogen fluoride yielding 11*a*-halo-6-methylenetetracyclines. The final step in this conversion includes the dehalogenation of 11*a*-halo-6-methylenetetracycline by reduction.

While the chlorination and fluorination of tetracycline have been the subjects of several investigations[1–4], a corresponding study of the bromination of tetracycline has never been described in detail. Thus, in the patent literature[1,3], 11*a*-bromotetracycline-6,12-hemiketal (II) is mentioned only as a highly reactive and water-sensitive substance.

We undertook the present investigation to examine the bromination and consequent dehydration of tetracycline in order to determine the convenience of using bromohemiketal II for the preparation of the corresponding 11*a*-bromo-6-methylenetetracycline (III) and 6-methylenetetracycline (VI).

We found that pure bromo-hemiketal II could be obtained from tetracycline (I) in 1,2-dimethoxyethane by bromination with bromine dissolved in CCl_4 in the presence of pyridine. This substance has spectral properties similar to that of the chloro analogue[2] and can be stored in the dark. An identical compound, but in lower yield, was obtained using *N*-bromosuccinimide as the brominating agent.

Although hydrogen fluoride was the preferred dehydrating agent[4] in this work sulfuric acid was found as a more convenient one.

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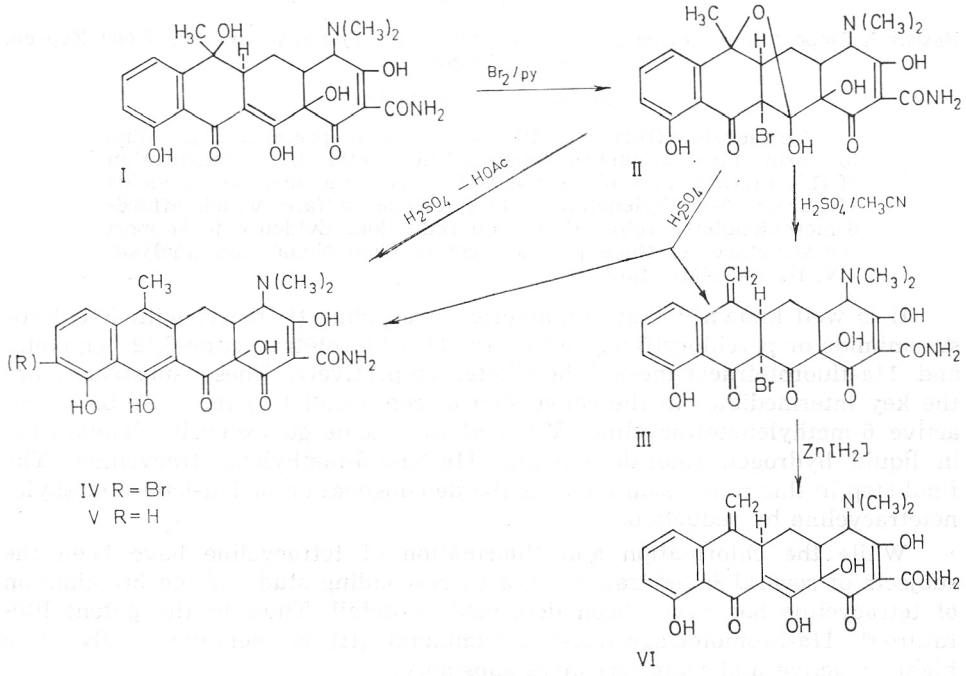
In attempting to dehydrate hemiketal II to the corresponding methylenetetracycline III using sulfuric acid, we encountered many difficulties. Namely, that the yield of III when using concentrated sulfuric acid as the dehydrating agent was very low. When 70% sulfuric acid was used, a mixture of bromotetracycline III and presumably 9-bromoanhydrotetracycline (IV) as the corresponding sulfates were isolated in crystalline forms, stable when stored in the dark. Experiments in which a mixture of sulfuric acid and acetic acid was used afforded the anhydro compound IV as the sole product. Finally, when hemiketal II was treated with a mixture of sulfuric acid and acetonitrile (1 : 1.4) an excellent yield of crystalline 11*a*-bromo-6-methylenetetracycline (III) was obtained as sulfate.



The structure of compound III was confirmed by its physical and chemical similarities to synthesized 11*a*-chloro-6-methylenetetracycline sulfate.* In addition, treatment of III as sulfate with zinc in methanol solution produced a high yield of fully biologically active 6-methylenetetracycline (VI). The UV, IR and NMR spectra of III are very similar and almost identical to those of the chloro analogue, thus supporting a like structure for these compounds. It is necessary to mention that the UV spectrum must be made immediately after the MeOH-HCl solution of the sample is prepared, because on standing this substance changes rapidly into a compound which probably has the anhydro structure.

The structural assignment for anhydro compound IV is based on elementary analysis, UV, IR and NMR spectra. Thus the UV spectrum shows maxima at

* Its spectral data are almost identical to those of the hydrochloride of 11*a*-chloro-6-methylenetetracycline.[2]

228, 275 and 433 nm, which is characteristic for anhydro derivatives of tetracyclines[5]. Two doublets in the NMR spectrum centered at 2.40 and 2.91 τ assigned two ortho aromatic protons, the bromine atom must therefore be attached to position C 7 or C 9. The slight differences in UV and IR spectral data of 7-bromoanhydrotetracycline[6] in comparison with the spectrum of compound IV indicate C 9 bromo substitution. It is worthwhile emphasising that the 9-bromoanhydrotetracycline (IV) can also be prepared by the reaction of anhydrotetracycline (V) with *N*-bromosuccinimide. As an analogy, the reaction of anhydrodedimethylaminotetracycline with *N*-bromosuccinimide is reported to give 9-bromoanhydrodedimethylaminotetracycline[7].

EXPERIMENTAL

UV spectra were measured on a Beckman model DU-2 ultraviolet spectrophotometer. IR spectra were recorded in potassium bromide pellets using a Perkin-Elmer Infracord model 137. NMR spectra were obtained in CF_3COOH solution using a Varian A-60*A* spectrometer and tetramethylsilane as an internal standard.

11a-Bromotetracycline-6,12-hemiketal (II)

a) Amphoteric, anhydrous tetracycline (I, 0.888 g, 2 mmoles) was dissolved in 1,2-dimethoxyethane (12 ml). The solution cooled to 5 °C was treated with pyridine (0.18 ml). After two minutes, 0.92 ml of a freshly prepared solution of bromine in tetrachloromethane (1 ml of bromine and 10 ml of tetrachloromethane) was added to this solution during period of 2 minutes. The reaction mixture was stirred at ice-bath temperature for a further 2 minutes, and 15 ml of cold water was added. After stirring for an additional 15 minutes, a solid separated, which was filtered off, washed with water and acetone, and dried under reduced pressure at room temperature. The yield was 0.43 g (41%) of a colorless crystalline product. UV spectrum: $\lambda_{max}^{MeOH—0.01\ N\ HCl}$ 268, 350 nm; log ε 4.29, 3.57.

Anal. $C_{22}H_{23}BrN_2O_8H_2O$ (541.37) calc'd.: C 48.81, H 4.65; N 5.17; Br 14.76%
found: C 48.32, H 4.85, N 5.15, Br 14.91%

b) *N*-Bromosuccinimide (0.178 g, 1 mmole) was added to a solution of amphoteric anhydrous tetracycline (I, 0.444 g, 1 mmole) in 1,2-dimethoxyethane (6 ml) at ice-bath temperature, and stirred for 7 minutes. The solid which separated was dissolved by the addition of cold water (6 ml). Stirring was continued for a further 30 minutes. The yield was 0.075 g (14%) of colorless crystals. UV and IR spectra were superimposable upon those of the product obtained as described under *a*).

11a-Bromo-6-methylenetetracycline (III) *as sulfate*

11*a*-Bromotetracycline-6,12-hemiketal (II, 0.541 g, 1 mmole) was gradually added to a stirred mixture of sulfuric acid (5 ml) and acetonitrile (7 ml) cooled to cca 5 °C. The solution was stirred in an ice-bath for 1 hour then diluted with *n*-butanol (50 ml) maintaining the temperature at 5—10 °C, and stirred for an additional 30 minutes. The solution was then allowed to stand overnight in a refrigerator. The product was filtered off, washed with acetone-ether (1:1) and dried in the dark. The yield of 11*a*-bromo-6-methylenetetracycline as sulfate containing one crystalline molecule of *n*-butanol was 0.510 g (75%). The product darkened on exposure to light.

For analysis 0.27 g of this material was dissolved in methanol (6 ml), and *n*-butanol (7 ml) was then added. On standing for several hours in a refrigerator, 0.215 g (80%) of a product consisting of pale yellow needles was obtained. UV spectrum: $\lambda_{max}^{MeOH—0.01\ N\ HCl}$ 237, 273, 377 nm; log ε 4.30, 4.24, 3.59 measured immediately after the solution was prepared. UV spectrum: $\lambda_{max}^{MeOH—0.01\ N\ HCl}$ 226, 273, and 420 nm measured after standing for 148 hours. IR spectrum: λ_{max}^{KBr} 5.72 nm (12-ketone). NMR spectrum: 3.94 and 4.35 τ (the methylene group).

Anal. $C_{22}H_{23}BrN_2O_{11}S$ BuOH (677.55)
calc'd.: C 46.09, H 4.91, N 4.13, Br 11.79%
found: C 45.97, H 5.00, N 4.30, Br 12.08%

9-Bromoanhydrotetracycline (IV) *as sulfate*

An equimolar amount of *N*-bromosuccinimide was added to a clear stirred solution of anhydrotetracycline[5] (V, 0.05 g, 0.117 mmoles) in 1,2-dimethoxyethane (3 ml). The reaction mixture was stirred at room temperature for 20 minutes. It first appeared as a dark green solution which after several minutes turned brown. 70% Sulfuric acid (0.2 ml) was added dropwise to the reaction mixture. During this time the precipitate which formed was redissolved. The solution was then diluted with water (1.5 ml) and allowed to stand, first at room temperature for 2 hours, and then in the refrigerator overnight. The resulting crystalline products (0.034 g, 48%) were collected by filtration and then dried.

For analysis this product was dissolved in *N,N*-dimethylformamide (1 ml) and precipitated as orange needles with dioxane (3 ml). UV spectrum: $\lambda_{max}^{0.1\ N\ HCl}$ 228, 275, 433 nm; log ε 4.50, 4.67, 3.88. NMR spectrum: 2.40 and 2.91 τ two doublets (ortho aromatic protons).

Anal. $C_{22}H_{23}BrN_2O_{11}S\ H_2O$ (621.44)
calc'd.: C 42.52, H 4.05, N 4.51, Br 12.86, S 5.16%
found: C 42.90, H 4.03, N 4.44, Br 12.67, S 5.25%

Treatment of 11a-Bromotetracycline-6,12-hemiketal with Sulfuric and Acetic Acid

a) 11*a*-Bromotetracycline-6,12-hemiketal (II, 0.200 g, 0.383 mmoles) was gradually added to a stirred solution of 70% sulfuric acid (2 ml) cooled to 10 °C. The reaction solution was stirred at this temperature for an additional 90 minutes, and then diluted with water (5 ml) maintaining the temperature between 10° and 15 °C. The resulting crude product (0.190 g) was filtered off and washed with water, and then with the mixture of acetone and ether (1 : 1).

Separation of 9-bromoanhydrotetracycline (IV) *as sulfate.* — The crude product obtained above was dissolved in *N,N*-dimethylformamide (1.5 ml), and precipitated by dioxane (5 ml.). The crystals thus separated (0.072 g, 31%) proved to be identical to 9-bromoanhydrotetracycline (IV) as sulfate obtained by the reaction of anhydrotetracycline (V) with *N*-bromosuccinimide. The UV and IR spectra of two materials were superimposable.

Separation of 11a-bromo-6-methylenetetracycline (III) *as sulfate.* — In another experiment the crude product was dissolved in methanol (7 ml) and filtered off. The filtrate was diluted with *n*-butanol (7 ml) and allowed to stand at room temperature for several hours. 11*a*-Bromo-6-methylenetetracycline (III) as sulfate (0.068 g, 26%) was recovered by filtration as pale yellow needles. Its UV and IR spectra were superimposable upon those of the compound III obtained by dehydration of hemiketal II with a mixture of sulfuric acid and acetonitrile.

b) 11*a*-Bromotetracycline-6,12-hemiketal (II, 0.2 g, 0.383 mmoles) was treated with concentrated sulfuric acid (2 ml) under the same conditions as above. From the reaction mixture 11*a*-bromo-6-methylenetetracycline (III) as sulfate was isolated in traces.

c) 11*a*-Bromotetracycline-6,12-hemiketal (II, 0.33 g, 0.63 mmoles) was gradually added with stirring to acetic acid (6 ml). The suspension was then stirred and cooled in an ice-bath and concentrated sulfuric acid (0.6 ml) was added to it dropwise. The clear deep-orange solution thus obtained was left to stand at room temperature overnight. The resulting product was collected, washed well with a mixture of acetone and ether (1 : 1) and dried. This material (0.290 g, 77%) was then dissolved in *N,N*-dimethylformamide and recrystallized as orange needles following the addition of dioxane. The infra-red spectrum of this product was identical to that of 9-bromoanhydrotetracycline (IV) as sulfate obtained by the reaction of anhydrotetracycline (V) with *N*-bromosuccinimide.

6-Methylenetetracycline (VI) *as hydrochloride*

Conversion of 11a-bromo-6-methylenetetracycline (III as sulfate, 0.200 g, 0.296 mmoles) to 6-methylenetetracycline (VI) was achieved by dissolving it in methanol (10 ml) with the addition of zinc (0.050 g) and stirring the mixture for 20 minutes at ice-bath temperature. After filtration and evaporation of methanol the crude product was suspended in stirred hot water (1.8 ml). Concentrated hydrochloric acid (0.06 ml) was then added and stirring was continued for 1 hour at room temperature. The product was filtered off and washed with cold 0.1% HCl and then with a mixture of acetone and ether (1 : 1). After drying at room temperature the pure product yielded 0.113 g (82%). It was recrystallized from hot diluted hydrochloric acid (0.3—1%) as glistening plates. Its UV and IR spectra were superimposable upon those of an original sample obtained by dehalogenation of 11a-chloro-6-methylenetetracycline hydrochloride.[2] Mixed m. p. 213—214 °C (dec.). (Lit.[8] 213.8—214.2 °C).

Anal. $C_{22}H_{23}ClN_2O_7$ H_2O (480.91) calc'd.: C 54.95, H 5.24, N 5.83, Cl 7.37%
found: C 55.10, H 5.33, N 5.60, Cl 7.37%

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IZVOD

Bromiranje tetraciklina

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Reakcijom tetraciklina (I) s bromom u prisutnosti piridina dobiven je 11a-brom-tetraciklin-6,12-hemiketal (II). Dehidratacijom hemiketala II u sumpornoj kiselini dobivena je smjesa od 11a-brom-6-metilentetraciklin sulfata (III) i 9-bromanhidro-tetraciklin sulfata (IV). Dehidratacija u smjesi sumporne i octene kiseline daje isključivo anhidro spoj IV. Brom-metilentetraciklin III kao čista tvar dobiven je dehidratacijom hemiketala II u smjesi sumporne kiseline i acetonitrila. Ovako dobiven spoj uspješno je preveden u biološki aktivan 6-metilentetraciklin (VI).

Novim spojevima (II, III i IV) određena je struktura na osnovu spektralnih podataka i elementarne analize.

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