INVESTIGATIONS IN THE FIELD OF TETRACYCLINES

LIV. Acid-Base Isomerizations of the Tetracyclines*

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Khimiya Prirodnykh Soedinenii, Vol. 6, No. 2, pp. 251–257, 1970

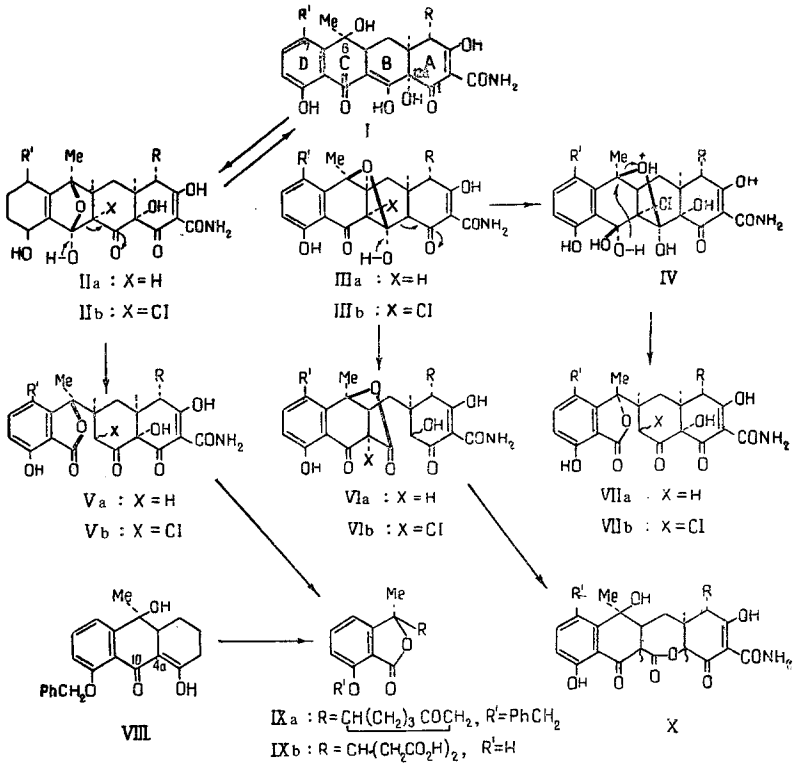
UDC 547.682.1+547.443

The tetracycline antibiotics are unstable at high and low pH values, undergoing various transformations under these conditions which involve the polycarbonyl system of the "lower periphery" of their molecule and the hydroxyl group in position 6. One of such transformations is the isomerization of the tetracyclines (I) under the action of aqueous alkalies into the isotetracyclines (V). This reaction consists formally of an intramolecular alcoholysis, and to explain it Woodward et al. [2] postulated the intermediate formation of 6,11-semiketals of type II. However, in a study of the similar transformation in a model hydroxydiketone (VIII), we found that this compound undergoes no change under the action of strong bases in aprotic solvents and the presence in the reaction medium of proton-donating molecules of the type of Z—H is necessary for its isomerization into the phthalide derivative IXa [3]. In view of this, we have studied the behavior of the tetracyclines themselves in the presence of bases in aprotic solvents. It was found that under the influence of a solution of NaH in dimethyl sulfoxide the tetracyclines I undergo isomerization of a different type, being converted into the γ-lactones VI. The same reaction was discovered independently and apparently somewhat earlier by Schwarz and Applegate, who heated tetracycline (I; R = NMe_2, R' = H) with triethylamine in dioxane and obtained the lactone VIa (R = NMe_2, R' = H), which they called allotetracycline [4]. It is quite obvious that the reaction takes place through the 6,12-semiketal IIIa, the direction of the retroaldol cleavage of the 12-hydroxy-1,11-diketone grouping being determined by the greater electron-accepting properties of the 1-oxo group than of the 11-oxo group. In our investigation of this transformation, we have found that in addition to the γ-lactones VIa, the isomeric ε-lactones X are also formed; we have called the latter pseudotetracyclines. They represent by-products formed by the intramolecular opening of the lactone ring in compounds VIa by the 12a-hydroxyl, and are sometimes isolated in the form of two stereoisomers (Xα and Xβ).

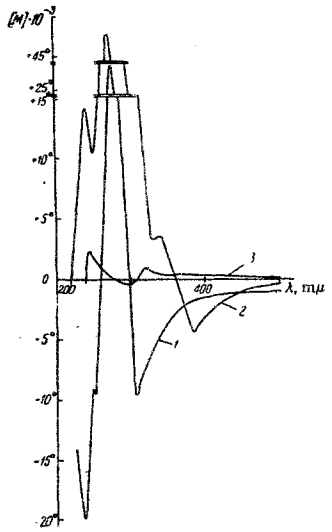
The rate of conversion of the tetracyclines I into the allo- and pseudo-compounds VIa and X depends markedly on the polarity of the solvent. Thus, in dioxane solution 15–20 hr is necessary for the isomerization of the tetracycline I (R = NMe_2, R = H) under the action of Et_3N at 100° C, while in dimethyl sulfoxide this reaction is complete in 5–10 min. The 4-desdimethylaminotetracycline I (R = R' = H) isomerizes similarly but considerably more slowly, while 4-desdimethylamino-12a-desoxytetracycline is so stable to the action of bases in atropic solvents that it does not isomerize even on being heated with NaH in Me_2SO for 10 hr. The cause of the increase in stability when the angular hydroxyl is removed is apparently the change in the direction of enolization of the 12-oxo group and, as a consequence, the loss of the capacity for the formation of a 6,12-semiketal. In view of this, the isomerization of 11a-halogenotetracyclines, which, in contrast to the 11a-unsubstituted tetracyclines (I), exist completely in the 6,12-semiketal form (IIIb) [5], appeared of interest. We have investigated the transformation of the semiketals for the case of the 7,11a-dichloro-4-des-dimethylamino derivative (IIIb; R = H, R' = Cl) since in this compound the absence of a NMe_2 group in ring A eliminates the possibility of 4-epimerization and the indeterminacy of the $C_{(4)}$ configuration associated with it, while the presence of chlorine in position 7 must facilitate the transformation into an isotetracycline (see [6]). It was found that the isomerization of compound IIIb (R = H, R' = Cl) under the action of bases and subsequent reductive dechlorination with zinc in acetic acid gave the allotetracycline VIa (R = H, R' = Cl) and did not form the corresponding isotetracycline (Va), regardless of whether isomerization was carried out in an aprotic medium (brief heating with Et_3N in Me_2SO) or in aqueous solution (with the aid of 0.5 N KOH). It followed from this that the fixing of the 6,12-semiketal structure III in the tetracyclines as the result of the introduction of halogen in position 11a practically deprives the molecule of the capacity for reacting in the tautomeric 6,11-semiketal form II and, thus, makes isomerization into the isotetracycline V impossible.

This conclusion was, however, contradicted by a paper reporting that 11a-chlorotetracycline is converted into

*For part LIII, see [1].


I
IIa : X=H
IIb : X=Cl
IIIa : X=H
IIIb : X=Cl
IV
Va : X=H
Vb : X=Cl
VIa : X=H
VIb : X=Cl
VIIa : X=H
VIIb : X=Cl
VIII
IXa : R=CH(CH2)3COCH2, R'=PhCH2
IXb : R=CH(CH2CO2H)2, R'=H
X

11a-chloroisotetracycline under the action of dilute HCl* [7]. Consequently, we subjected compound IIIb (R = H, R' = Cl) to acid isomerization and then to reductive dechlorination. A substance was obtained which proved not to be identical with the product of the alkaline isomerization of the 11a-unsubstituted compound I (R = H, R' = Cl). The optical rotatory dispersion of this compound shows that it differs from isotetracycline (Va; R = H, R' = Cl) in the configuration of the $C_{(6)}$ asymmetric center, i.e., it is 6-epiisotetracycline (VIIa; R = H, R' = Cl). In actual fact, a comparison of the ORD curves of the two isomerization products and a model S-(+)- compound (IXb) [9] (figure) permits the conclusion that the main contribution to the optical activity at 260–320 mμ is made by the structure of rings A and B. The rotation due to the phthalide chromophore appears most strongly below 250 mμ and in this region the isotetracycline Va (R = H, R' = = Cl) and the acid IXb have negative Cotton effects and the 6-epiisotetracycline VIIa (R = H, R' = Cl) a positive one.



Optical rotatory dispersion in methanol: 1) 7-chloro-4-desdimethylaminoisotetracycline (Va); R = H, R' = Cl; 2) 7-chloro-4-desdimethylamino-6-epiisotetracycline (VIIa), R = H, R' = Cl; 3) S-(+)-β-(4-chloro-7-hydroxy-3-methylphthalid-3-yl)glutaric acid (IXb).

Thus, the isomerization of the 11-halogenotetracyclines under the action of acids is accompanied by the inversion of the $C_{(6)}$ asymmetric center and leads to previously unknown compounds, derivatives of 6-epiisotetracycline. It is likely that this transformation begins with the hydration of the 11-oxo group and with intramolecular nucleophilic substitution at $C_{(6)}$ in the $O_{(6)}$-protonated form (IV); the resulting 6,11-semiketal (6-epi-11b) then undergoes a retroaldol cleavage at the $C_{(11)}$—$C_{(11a)}$ bond.

EXPERIMENTAL

All the UV spectra were taken in 96% ethanol, the IR spectra in mulls with paraffin oil. Chromatography was carried out in a nonfixed layer of silica gel of the "vodnaya kremnevaya kislota" ["aqueous silicic acid"] type (activity grade II) in ethyl acetate–heptane systems and on paper of the "fast" type impregnated with Trilon B in the butan-1-ol–4% aqueous NH_3, upper phase, system. The molecular weights were determined by mass spectrometry. The IR spectra were taken by L. B. Sinyavina and the optical rotatory dispersions were measured by G. A. Kogan and M. I. Struchkova.

1. Allotetracycline (VIa: R = NMe_2, R' = H) and the stereoisomeric pseudotetracyclines X(R = NMe_2, R' = H). A) A solution of 3.9 g of tetracycline (I; R = NMe_2, R' = H) and 0.93 g of triethylamine in 20 ml of dioxane was heated at the boil in an atmosphere of argon for 18 hr, and then the solvent was distilled off in vacuum, the residue was dissolved in 20 ml of acetone with boiling, 15 ml of benzene was added, and the solution was evaporated to a volume of 20 ml. This gave 2.4 g (62%) of a mixture of allotetracycline (VIa) and pseudotetracyclines (X), from which the

* Under the action of concentrated acids, 11a-halogenotetracyclines are dehydrated to 6, 13-anhydrotetracyclines [7,8].

allotetracycline was isolated by recrystallization from benzene. mp 131° C; R_f 0.55 (on silica gel, 1 : 1); R_f 0.13 and 0.30 (on paper); λ_{max}, mμ: 265, 340 (log ε 4.09, 3.39); ν_{max}, cm^{-1}: 3400, 1770, 1645, 1572; $[\alpha]_{589}^{26}$ −140°, $[\alpha]_{393}$ 0°, $[\alpha]_{365}$ +160°, $[\alpha]_{330}$ −1000°, $[\alpha]_{290}$ +2300° (c 0.1; MeOH).

Judging from the results of chromatography on silica gel, the mother solution contained a mixture of allotetracycline (VIa) and pseudotetracyclines (X) (see Experiment 1C).

B) A solution of 0.58 g of tetracycline in 2 ml of dimethyl sulfoxide was treated with 39 mg of 80% sodium hydride. The mixture was heated at 100° C for 10 min and was then diluted with 5 ml of water and extracted with ethyl acetate. This gave 400 mg (69%) of a mixture of allotetracycline (VIa) and pseudotetracycline (X).

C) To a solution of 0.60 g of allotetracycline VIa; (R = NMe_2, R' = H) in 5 ml of dimethyl sulfoxide was added 40 mg of 80% sodium hydride. The mixture was heated at 100° C for 5 hr and was treated in a similar manner to experiment B. This gave 150 mg of a mixture of pseudotetracyclines (X; R = NMe_2, R' = H), which were separated by chromatography on silica gel (1 : 1). One of the isomers (Xα) had R_f 0.70; λ_{max}, mμ: 265, 365 (log ε 4.47, 3.95); ν_{max}, cm^{-1}: 3400, 1740, 1621, 1570; $[\alpha]_{589}^{26}$ −160°, $[\alpha]_{345}$ −1600°, $[\alpha]_{325}$ 0°, $[\alpha]_{305}$ +2400°, $[\alpha]_{275}$ −2100° (c 0.2; MeOH). The second isomer (Xβ) and R_f 0.55; λ_{max}, mμ 263, 310, 362 (log ε 4.28, 3.91, 4.08); ν_{max}, cm^{-1}: 3400, 1740, 1621, 1570; $[\alpha]_{589}^{26}$ −120°, $[\alpha]_{345}$ −6400°, $[\alpha]_{310}$ 0°, $[\alpha]_{295}$ +4000°, $[\alpha]_{280}$ 0° (c 0.2; MeOH).

2. 7-Chloroallotetracycline (VIa; R = NMe_2, R' = Cl). 7-Chlorotetracycline (I; R = NMe_2, R = Cl) (0.69 g) was isomerized under the conditions of Experiment 1B. This gave 0.45 g (65%) of 7-chloroallotetracycline (VIa), which was purified by chromatography on silica gel (1 : 1). mp 162° C; R_f 0.60 (on silica gel, 1 : 1); R_f 0.13 and 0.30 (on paper); λ_{max}, mμ: 265, 320, 365 (log ε 4.33, 3.84, 3.89); ν_{max}, cm^{-1}: 3360, 1770, 1640, 1575; $[\alpha]_{589}^{26}$ −150°, $[\alpha]_{410}$ −620°, $[\alpha]_{345}$ 0°, $[\alpha]_{300}$ +1760° (c 0.1; MeOH).

3. 4-Desdimethylaminoallotetracycline (VIa; R = R' = H) and 4-des-dimethylaminopseudotetracycline (X; R = = R' = H). A) A solution of 300 mg of 4-desdimethylaminotetracycline (I; R = R' = H) in 2 ml of dimethyl sulfoxide was treated with 30 mg of 80% sodium hydride, and the mixture was heated at 100° C for 5 hr and was then treated as in Experiment 1B. This gave 300 mg of a mixture of compounds I, VIa, and X, which were separated by repeated chromatography on silica gel (1 : 1). The zone with R_f 0.60 yielded 20 mg of 4-desdimethylaminoallotetracycline (VIa; R = R' = H); λ_{max}, mμ: 265, 335 (log ε 4.54, 3.95); ν_{max}, cm^{-1}: 3400, 1770, 1610, 1595; $[\alpha]_{370}^{26}$ 0°, $[\alpha]_{360}$ +100°, $[\alpha]_{325}$ −400°, $[\alpha]_{284}$ +420°, $[\alpha]_{260}$ 0° (c 0.1; MeOH).

The zone with R_f 0.50 yielded 30 mg of 4-desdimethylaminopseudotetracycline (X; R = R' = H); λ_{max}, mμ: 258, 310 (log ε 3.98, 3.73); ν_{max}, cm^{-1}: 3350, 1720, 1650, 1600; $[\alpha]_{380}^{26}$ −200°, $[\alpha]_{340}$ −400°, $[\alpha]_{300}$ +500°, $[\alpha]_{272}$ +175°, $[\alpha]_{260}$ +240°, $[\alpha]_{243}$ 0° (c 0.1; MeOH).

B) A solution of 300 mg of allotetracycline (VIa; R = NMe_2; R' = H) in 3 ml of tetrahydrofuran was treated with 0.8 ml of methyl iodide. The mixture was kept at room temperature for 8 days and was then evaporated and the residue was triturated with ethyl acetate and filtered off. This gave 240 mg of allotetracycline methiodide, which was dissolved in 10 ml of 50% acetic acid, and the solution was treated with 130 mg of zinc dust and stirred for 15 min. Then it was filtered, acidified with 0.4 ml of conc HCl, and extracted with ethyl acetate. This yielded 4-des-dimethylaminoallotetracycline (VIa; R = R' = H), which has been described in Experiment 3A, and this was purified by chromatography on silica gel. Yield 47 mg (20%).

4. 7,11a-Dichloro-4-desdimethylaminotetracycline (IIIb; R = H, R' = Cl). A solution of 440 ml of 7-chloro-4-desdimethylaminotetracycline (I; R = H, R' = Cl) [2] in 5 ml of tetrahydrofuran was treated with 133 mg of N-chlorosuccinimide in 3.5 ml of the same solvent. The mixture was left at room temperature for 1 hr, and was then evaporated and the residue was chromatographed on silica gel (1 : 1). The zone with R_f 0.6–0.75 yielded 405 mg (85%) of the 11a-chloro derivative IIIb (R = H, R' = Cl). mp 121–123° C; R_f 0.67 (1 : 1); λ_{max}, mμ: 267, 357 (log ε 4.36, 3.64); ν_{max}, cm^{-1}: 3400, 1740, 1715, 1650, 1630, 1580; $[\alpha]_{589}^{26}$ 0°, $[\alpha]_{436}$ −200°, $[\alpha]_{390}$ −720° (c 0.1; MeOH).

Found, mol wt 469. Calculated for $C_{20}H_{17}^{35}Cl_2NO_8$: mol wt 469.

5. 7-Chlorodesdimethylaminoallotetracycline (VIa; R = H, R' = Cl). A) A solution of 500 mg of 7,11a-dichloro-4-desdimethylaminotetracycline (IIIb; R = H, R' = Cl) in 7 ml of ethanol was treated with 7 ml of 1 N KOH, and the mixture was kept at room temperature for 4 hr and was then acidified with 1 N H_2SO_4 to pH 5 and extracted with ethyl acetate, and the extract was dried with Na_2SO_4 and evaporated. The residue (250 mg) was dissolved in 10 ml of acetic

acid and the solution was treated with 0.5 g of zinc dust, stirred for 1 hr, filtered, and evaporated. The residue was chromatographed on silica gel (1 : 2), the zone with R_f 0.1–0.2 being isolated. This gave 28 mg (6%) of chlorodesdimethylallotetracycline (VIa; R = H, R' = Cl). mp 110–115° C (from benzene); R_f 0.13 (1 : 2); λ_{max}, mμ: 260, 320 (log ε 4.37, 3.92); ν_{max}, cm^{-1}: 3170, 3080, 1778, 1700, 1620, 1565; $[\alpha]_{589}^{26}$ −30°, $[\alpha]_{342}$ −523°, $[\alpha]_{300}$ +508°, $[\alpha]_{280}$ +1120°, $[\alpha]_{248}$ −328°, $[\alpha]_{235}$ +149°, $[\alpha]_{217}$ −746° (c 0.1; MeOH).

B) A solution of 500 mg of compound IIb (R = H, R' = Cl) and 360 mg of triethylamine in 5 ml of dimethyl sulfoxide was heated at 100° C for 1 hr and after cooling it was acidified with 1 N H_2SO_4 to pH 5 and extracted with ethyl acetate. The further treatment was carried out as in Experiment 5A. This yielded 24 mg (5%) of the allo derivative VIa (R = H, R' = Cl) described in experiment 5A.

6. 7-Chloro-4-desdimethylamino-6-epiisotetracycline (VIIa; R = H, R' = Cl). A solution of 300 mg of the 11a-chloro derivative IIIb (R = H, R' = Cl) in 7.5 ml of ethanol was treated with 1.5 ml of conc HCl, the mixture was heated to boiling for 4 hr and the alcohol was distilled off in vacuum. The residue was extracted with ethyl acetate, and the extract was washed with water, dried with Na_2SO_4, and evaporated. The substance obtained was dissolved in 5 ml of acetic acid and the solution was treated with 0.5 g of zinc dust, stirred vigorously at 20° C for 15 min, filtered, and evaporated, and the residue was chromatographed on silica gel (1 : 2). The zone with R_f 0.25–0.35 yielded 19 mg (7%) of 6-epiisotetracycline (VIIa; R = H, R' = Cl). mp 175–178° C (from benzene); R_f 0.31 (1 : 2); λ_{max}, mμ: 220, 267, 320, 362 (log ε 4.38, 4.37, 3.51, 3.64); ν_{max}, cm^{-1}: 3400, 3300, 1745, 1647, 1630, 1580; $[\alpha]_{589}^{26}$ −52°, $[\alpha]_{385}$ −1000°, $[\alpha]_{341}$ +800°, $[\alpha]_{330}$ +760°, $[\alpha]_{273}$ +10 800°, $[\alpha]_{250}$ +3000°, $[\alpha]_{220}$ 0° (c 0.1; MeOH).

Found, mol wt 435. Calculated for $C_{20}H_{18}^{35}ClNO_8$: mol wt 435.

7. 7-Chloro-4-desdimethylaminoisotetracycline (Va; R = H, R' = Cl) was obtained by the alkali isomerization of 7-chloro-4-desdimethylaminotetracycline (I; R = H, R' = Cl) by the method of Stephens et al. [2]. mp 260° C (decomp., from benzene); R_f 0.24 (on silica gel, 1 : 2); λ_{max}, mμ: 240, 267, 315, 355 (log ε 4.22, 4.23, 3.72, 3.37); ν_{max}, cm^{-1}: 3460, 3415, 3310, 1760, 1725, 1640, 1630, 1575; $[\alpha]_{589}^{26}$ −110°, $[\alpha]_{370}$ −570°, $[\alpha]_{308}$ −2200°, $[\alpha]_{275}$ +6200°, $[\alpha]_{252}$ −2200°, $[\alpha]_{250}$ −2100°, $[\alpha]_{238}$ −4600°, $[\alpha]_{228}$ −3200° (c 0.1; MeOH).

Found: mol wt 435. Calculated for $C_{20}H_{18}^{35}ClNO_8$: mol wt 435.

8. 3-(4'-Chloro-7'-hydroxy-3'-methylphthalid-3'-yl)glutaric acid (IXb) was obtained from the corresponding 7'-methoxy- acid [9] by heating it with a 20-fold amount of 48% HBr (3 hr at the boil), and was isolated by chromatography on silica gel (1 : 1). Yield 60%; R_f 0.42; mp 74–76° C; λ_{max}, mμ: 228, 243, 316, 352 (log ε 4.11, 3.77, 3.52, 3.53, 3.33). ν_{max}, cm^{-1}: 3300–2600, 1748, 1728, 1712, 1620; $[\alpha]_{589}^{26}$ +40°, $[\alpha]_{320}$ +300°, $[\alpha]_{310}$ 0°, $[\alpha]_{295}$ −100°, $[\alpha]_{280}$ 0°, $[\alpha]_{245}$ +700°, $[\alpha]_{240}$ 0° (c 0.1; MeOH)

Found: mol wt 328, Calculated for $C_{14}H_{13}^{35}ClO_7$: mol wt 328.

CONCLUSIONS

The direction and mechanism of the isomerization of the tetracyclines (I) into the allotetracyclines (VI) and pseudotetracyclines (X), isotetracycline (V), and 6-epiisotetracycline (VII) are determined by intramolecular 6,11- or 6,12-ketalization which, in its turn, depends on the structure of the initial tetracyclines and the composition of the medium.

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8 August 1969

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