CHEMICAL MODIFICATIONS IN THE TETRACYCLINE SERIES

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New tetracycline analogs modified at position 5, 6 and 2 were synthetized. The 5-deoxy-5-oxo-derivatives, **2a** and **3a**, were obtained by DMSO/acetic anhydride oxidation of doxycycline (**2**) and methacycline (**3**), respectively; the 6-demethyl-6-hydroxymethyl-6-alpha-hydroxy-oxytetracycline (**3b**) by methacycline oxidation with the $KClO_3/OsO_4$ system and the 6-hydroxy-anhydrooxytetracycline (**4**) treating **3b** with periodic acid. The 2-ethoxycarbonyl-2-de-carboxamidodoxycycline (**2b**), was synthetized by treating doxycycline nitrile (**2c**) with EtOH and anhydrous HCl, 2-thiocarboxamide-2-decarboxamidodoxycycline (**2d**) by reaction of doxycycline with P_2S_5 in dioxane and 2-aminomethyl-2-decarboxamidodoxycycline (**2e**) by RANEY-Nickel reduction of **2d**. All the synthetized compounds proved to be almost inactive on agar plates both on Gram-positive and Gram-negative bacteria.

Tetracycline antibiotics continue also today to play an important role in human and veterinary medicine and in animal nutrition.

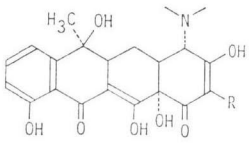
The greatest success in the development of new active tetracycline has been up to now obtained by modifications at the position 6. The position 5 also seems not very critical for antimicrobial activity influencing mainly the pharmacokinetics properties[1,2].

During a research program aimed at finding new and possibly clinically useful tetracycline derivatives, our synthetic efforts followed mainly two routes: on one side we took up the synthesis of some new derivatives (compounds **2a**, **3a**, **3b**, **4**) modified at position 5 and 6 starting from the easily available, acid stable doxycycline (**2**) and methacycline (**3**) and, on the other side, we paid also our attention to modifications at position 2.

From structure-activity relationships studies it is in fact shown that only a carbonyl substituent at position 2 and not the whole carboxamide group is essential for maintenance of activity: the nitrile (**1a**)[3] has no activity but derivatives bearing at the 2 positions an aldehyde (**1b**)[4], aldimine (**1c**)[5] or acetyl (**1d**)[6] group retain a certain degree of antimicrobial activity.

It seemed therefore interesting[7], in order to define the role of the C-2 carboxamidic group, to synthetize new C-2 derivatives, namely the 2-ethoxycarbonyl (**2b**), the 2-thiocarboxamide (**2d**), the 2-aminomethyl (**2e**) and the 2-N-methylaminomethyl doxycycline.

Fig. 1.



1 R=$CONH_2$ (tetracycline)
1a R=$C\equiv N$
1b R=CHO
1c R=CH=NOH
1d R=$COCH_3$

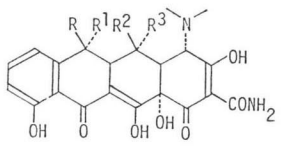
Results and Discussion

Modifications at C5

Pursuing the goal of obtaining new, previously unknown derivatives, we studied the oxidation of the 5-alpha-hydroxyl group to 5-keto of the two widely used semisynthetic tetracyclines, doxycycline (**2**) and methacycline (**3**). While the oxidation of **2** or **3** with chromium and manganese compounds led only to degradated products, reaction with dimethylsulfoxide (DMSO) and acetic anhydride gave mainly a product to which was assigned the structure **2a** or **3a** respectively.

The IR spectrum of these compounds was characterized by a peak at 1740 cm^{-1} according with a keto group not involved in the keto-enol system. The presence of an acetyl ester group was excluded by the NMR spectrum and also by some preliminary proofs in which the use of acetic anhydride was avoided and the reaction was carried out with DMSO and phosphoric acid giving the same product but in lower yield.

Fig. 2.



2 R=H, $R^1=CH_3$, R^2=H, R^3=OH (doxycycline)
2a R=H, $R^1=CH_3$, R^2, R^3=O
3 R, $R^1=CH_2$, R^2=H, R^3=OH (methacycline)
3a R, $R^1=CH_2$, R^2, R^3=O
3b $R=CH_2OH$, R^1=OH, R^2=H, R^3=OH

Both compounds **2a** and **3a** proved to be practically inactive when tested for minimal inhibitory concentration on agar plates, using a representative number of Gram-positive and Gram-negative bacterial strains normally used for screening tests.

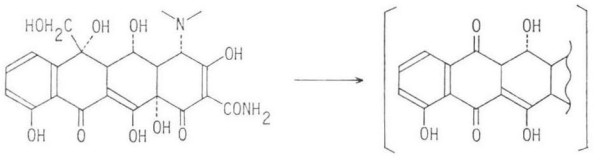
Modifications at C-6

Few reactions have been reported on the exocyclic double bond of the methacycline other than, a free-radical mercaptan addition[8] and catalytic hydrogenation.[9] According to our results, this double bond is very scarcely reactive, for example it has been impossible to epoxidize it without complete degradation of the molecule and it did not react at all with some methylides in anhydrous THF after complete sylilation of the hydroxyl groups. Nevertheless **3** reacts with the oxidizing system OsO_4/$KClO_3$ in MeOH/H_2O giving the 6-demethyl-6-hydroxymethyloxytetracycline (**3b**) in good yield. In the NMR spectrum of **3b** the shift of the CH_2 signal from δ 5.2 (in methacycline) to δ 3.4 (assigned to the CH_2OH) was diagnostic, nevertheless, it was impossible to assign the stereochemistry of the new asymmetric center only on this basis. Moreover **3b** when treated with refluxing 0.8 N hydrochloric acid in the usual way[10], didn't give the corresponding expected 5a-6-anhydroderivative, probably owing to a *cis*-correlation between the hydroxy group at C_5 and the hydrogen at C_{5a}. Also some speculations on molecular models about the side of OsO_4 addition were in agreement with an unnatural configuration at C_6.

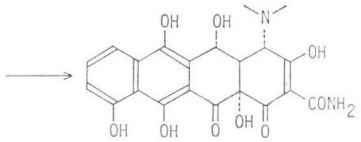
Compound **3b** reacted with periodic acid, in the usual mild conditions used for cleavage of 1,2-diols, to give the 6-demethyl-6-hydroxyanhydrooxytetracycline (**4**) *via* a labile 6-demethyl-6-oxodoxycycline. The structure of **4*** was mainly based on the UV spectrum on which the absorption at 274 nm (ε=16,000) indicated the A ring unchanged and a second band at 471 nm (ε=7,000) was considered diagnostic for the new B-C-D ring system, considering a *circa* 30 nm bathochromic shift[11] owing to the presence of the para hydroxyl group on the B ring, in comparison with the usual anhydrotetracycline derivative.

* In the same conditions, doxycycline did not react.

Scheme 1.

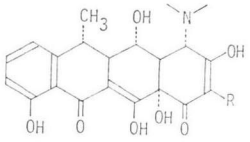


3b



4

Fig. 3.



2 R=$CONH_2$ (doxycycline)
2b R=COOEt
2c R=C≡N
2d R=$CSNH_2$
2e R=CH_2NH_2
2f R=$CONHCH_2N(CH_3)CH_2Ph$

Compound **3b**, when tested against some bacteria on agar plate (MIC) was shown to be less active than the parent tetracycline while **4** proved to be inactive.

Modifications at C-2

Doxycycline nitrile (**2c**), starting material for the 2-ethoxycarbonyl derivative (**2b**), has been prepared by reaction of doxycycline with dicyclohexylcarbodiimide (DCC) in MeOH (see Experimental).

Reaction of **2c** with hydrogen chloride in EtOH at reflux gave, after purification, in poor yields, the 2-ethoxycarbonyl-2-decarboxamidodoxycycline (**2b**). The structure of this compound was assigned on the base of its spectral data: the UV spectrum showed an unchanged doxycycline skeleton, the presence of the ethyl groups was diagnostic in the NMR, and in the IR spectrum the disappearance of the nitrile absorption band and the appearance of new peak at 1740 cm^{-1}, characteristic for an ester group, was clearly evident.

After many inconclusive efforts to obtain the 2-thioamide (**2d**) by treatment of the nitrile (**2c**) with H_2S or with thioacetamide, we found that the reaction of doxycycline hydrochloride with P_2S_5 in dioxane for 16 hours at room temperature, followed by purification, gave the 2-thiocarboxamido-2-decarboxamido-doxycycline (**2d**) in good yield.

The structure of **2d** was confirmed by elemental analysis, spectroscopic evidences and also by some chemical proofs.

So, the doxycycline nitrile (**2c**) did not react with P_2S_5, that is only the carboxamido group was involved in the reaction, and **2d** did not react, like doxycycline does, with dicyclohexylcarbodiimide (DCC) in methanol to give the corresponding nitrile derivatives (see Experimental).

The compound (**2d**) was also reduced with freshly prepared RANEY-Nickel to give the 2-aminomethyl derivative (**2e**). Also the structure of **2e** was assigned on the bases of the analytical data (absence of S in the elemental analysis and UV spectrum unchanged) and chemical reactions (doxycycline did not react in the same conditions).

Nevertheless, **2e** was not very stable, in our opinion owing to a SCHIFF intermolecular condensations of the new primary amino group.

A similar attack was also supposed the first step of a more complex rearrangement that has been observed in the attempted synthesis of the 2 N-methylaminomethyl derivative, during the benzyl group hydrogenolysis on Pt_2O of the compound **2f** obtained by a MANNICH reaction[12] among doxycycline,

formaldehyde and N-methyl-N-benzylamine*. While doxycycline with Pt_2O under hydrogen at atmospheric pressure did not react for several hours, in the case of **2f** the production near quantitative of toluene was observed (G.C.), but it was impossible to isolate the 2 N-methylaminomethyldoxycycline owing to further reactions of the secondary amino group to give a yellow-orange product not certainly identified on the basis of spectral data.

According to the NMR spectra of all these compounds modified at C-2, the asymmetric centers at C_4, C_{4a}, C_5, C_{5a}, C_6, C_{12a} remained unchanged during these reactions, nevertheless when tested for microbiological activity all the compounds were found practically inactive in respect to the parent doxycycline; only **2f** of course, maintains a good activity as the other derivatives of its type.

According to our present results it is possible to confirm that not only the carbonyl but all the carboxamido group at C-2 position is very important for the antibiotic activity and any modification which involves more than one hydrogen on it, leads to gaps in the antimicrobial spectrum. The diketo-amide moiety in the A ring is involved in a very unusual ketoenol tautomerism with very strong hydrogen bond. This fact is also evident in the NMR spectrum of doxycycline in which the two NH amide protons are quite different in the chemical shift (δ 8.1 and 8.8 DMSO-d_6). The chemical modifications carried out on compounds **2b, c, d, e** displace dramatically the extensive electron delocalization of this system (causing the lack of antibiotic activity) also in the case of **2d** as monitored by the NMR spectrum of this compound in which the NH_2 amide signal is a single peak at 9.2 indicating an electronic equivalence for the two protons.

Conclusions

Positions 5, 6 and also 2 in lesser extent, are known as not very critical for antibiotic activity (of course with marked differences between positions 5 and 6 which can tolerate even drastic modifications without significantly loosing antimicrobial potency and position 2 where a carbonyl group is said to be the minimum requirement for an antimicrobial action of no practical interest). The synthesis of new tetracycline analogs modified at these positions allows to confirm, and partially to extend, what is presently known about structure-activity relationships in the tetracycline field.

All the described compounds were practically inactive against both Gram-positive and Gram-negative bacteria. It is therefore possible to conclude that at C-2 the presence of an unchanged carboxamido group is very important and critical for maintenance of a significant and practically useful, antimicrobial action, and the hybridization of the 5 carbon atom cannot be changed.

More surprising was the poor activity of the 6-hydroxymethyl derivative (**3b**) (in every case the most active of the series studied), perhaps imputable to the unnatural stereochemistry at C-6.

Experimental

The melting points were uncorrected. ^1H NMR spectra were obtained at 60 MHz on a Perkin-Elmer R24-B spectrometer. Chemical shifts (δ) are reported as parts per million from tetramethylsilane. Infrared and ultraviolet spectra were recorded using a Perkin-Elmer 157 G and 550 spectrophotometer, respectively. Thin-layer chromatography (tlc) was carried out on Merck silica gel plates, sprayed with a 0.5% EDTA solution and dried at 110°C eluted with Me_2CO - EtOAc - H_2O (80: 35: 15). Column chromatographies were performed on silica gel (Merck, 60 ~ 200 mesh) pretreated with EDTA at pH 7.2 and dried at 110°C (eluted with Me_2CO - EtOAc - H_2O (80: 35: 15)) or on cellulose (eluted with *n*-BuOH

* The reaction was carried out as in Reference 12).

saturated with H_2O).

5-Deoxy-5-oxodoxycycline (**2a**)

A solution of doxycycline (8.8 g, 19.7 mmoles) in DMSO (40 ml) and Ac_2O (42 ml) was stirred for 20 hours at room temperature, then was diluted with water and extracted with *n*-BuOH (3×200 ml). The organic phase was dried on Na_2SO_4 and concentrated under vacuum. The residuum, diluted with MeOH (~10 ml), was precipitated with Et_2O. Purification on column chromatography (silica gel) of the crude yellow product (6 g, 68% yield) gave the pure 5-deoxy-5-oxodoxycycline (**2a**), m.p. 193~195°C; UV λ_{max}^{MeOH} 275 nm (ε=18,000), 365 (14,500); IR (nujol): 3300, 1740, 1660, 1620 cm^{-1}; NMR (DMSO-d_6): δ 1.2 (3H, d, CH_3), 2.4 (7H, m, $N(CH_3)_2$, C_6–H), 2.8 (2H, m, C_{4a}–H, C_{5a}–H), 3.3 (1H, d, C_4–H), 6.5~7.4 (3H, m, aromatics), 8.1~8.8 (2H, d, $CONH_2$). Compound (**3a**), a product with very similar analytical data, was obtained starting from **3** in similar way.

2-Ethoxycarbonyl-2-decarboxamidodoxycycline (**2b**)

A solution of 2-cyano-2-decarboxamidodoxycycline (5 g, 11.7 mmoles) in EtOH (150 ml) was saturated with anhydrous HCl and refluxed for 36 hours. The solvent was then evaporated and the residue diluted with water, neutralized to pH 4 and extracted in *n*-BuOH (3×100 ml). Usual work up and purification by column chromatography (silica gel) gave **2b** (1 g, 18% yield): m.p. 188~190°C; IR (nujol) 1730, 1620 cm^{-1}: UV λ_{max}^{MeOH} 265 nm (ε=17,700), 360 (ε=13,500); NMR (CF_3COOH): δ 1.1 (3H, d, CH_3–C_6), 1.6 (3H, t, $C\underline{H}_3$–CH_2–O), 2.7 (6H, s, $N(CH_3)_2$), 3.1~3.6 (3H, m, C_6–H, C_{5a}–H, C_{4a}–H), 3.7~4.1 (3H, m, C_4–H, CH_2), 5.0 (1H, d, C_5–H), 6.2~7.0 (3H, m, aromatics); $[\alpha]_D$+35.3° (*c* 0.02, MeOH).

2-Cyano-2-decarboxamidodoxycycline (**2c**)

A solution of doxycycline hydrochloride (50 g, 0.1 moles) and N,N-dicyclohexylcarbodiimide (DCC) (50 g, 0.24 moles) in MeOH (500 ml) was stirred for 6 hours at room temperature. The resulting solution was concentrated under vacuum and the dicyclohexylurea was filtered off. The clear solution was evaporated to give a crude product which was suspended in $CHCl_3$-Et_2O (2:1) and filtered. The yellow product (41 g) was crystallized from *n*-BuOH obtaining 23 g (51% yield) of **2c**. m.p. 226~228°C, IR (nujol) 2200, 1620 cm^{-1}; UV λ_{max}^{MeOH} 270 nm (ε=18,100), 360 (13,000) $[\alpha]_D$−74.01° (*c* 0.05, MeOH).

2-Thiocarboxamide-2-decarboxamidodoxycycline (**2d**)

A suspension of doxycycline hydrochloride (5 g, 10.4 mmoles) and P_2S_5 (2.87 g, 12.5 mmoles) in dioxane (100 ml) was stirred for 18 hours at 22°C. The resulting yellow solution was poured in cooled water (5°C), the pH adjusted to 4, filtered and the clear solution extracted with *n*-BuOH (3×200 ml). The organic layer, dried on Na_2SO_4, was evaporated *in vacuo* and the residue purified on column chromatography (silica gel). The yellow product was crystallized from MeOH (2.8 g, yield 58.3%): m.p. 222~225°C; IR (nujol) 3340, 1620 cm^{-1}; UV λ_{max}^{MeOH} 270 nm (ε=18,000), 360 (14,000); NMR (CF_3-COOH): δ 1.1 (3H, d, CH_3–C_6), 2.7 (6H, s, $N(CH_3)_2$), 3.1~3.6 (3H, m, C_6–H, C_{5a}–H, C_{4a}–H), 3.9 (1H, d, C_4–H), 4.8 (1H, d, C_5–H), 6.2~7.0 (3H, m, aromatics), 9.1 (2H, s $CSNH_2$); $[\alpha]_D$−180.33° (*c* 0.1, MeOH);

Anal. Calcd for $C_{22}H_{23}O_7N_2S$: C, 57.5; H, 5.04; N, 6.1; S, 6.98
Found: C, 56.8; H, 5.0; N, 5.9; S, 6.62

2-Aminomethyl-2-decarboxamidodoxycycline (**2e**)

A solution of **2d** (2.0 g, 4.3 mmoles) in EtOH (40 ml) was stirred under H_2 at 22°C with freshly prepared RANEY-nickel (10 g) until disappearance of **2d** (tlc monitoring). The catalyst was filtered off, the resulting solution concentrated *in vacuo* and the crude product crystallized from MeOH-Et_2O to give **2e** (1.6 g; yield 86%): m.p. 212°C (dec.); IR (nujol) 3340, 1620 cm^{-1}; UV λ_{max}^{MeOH} 225 nm (ε=16,200), 360 (12,000).

Anal. Calcd for $C_{22}H_{25}O_7N_2$: C, 61.5; H, 5.9; N, 6.52.
Found: C, 60.6; H, 5.6; N, 6.14.

6-Demethyl-6-hydroxymethyloxytetracycline (**3b**)

To a solution of methacycline hydrochloride (10 g, 22.5 mmoles) in H_2O (280 ml) and MeOH (150 ml) was added $KClO_3$ (5.5 g, 45 mmoles) and a catalytic amount of OsO_4. The solution was

stirred for 24 hours at room temperature then MeOH saturated with H_2S (250 ml) was added. The OsS_2 was filtered off and the MeOH concentrated under vacuum; the resulting aqueous solution, neutralized with 0.1 N NaOH and diluted with brine, was extracted with *n*-BuOH from which, after drying and concentration under vacuum, a yellow product was obtained (9.8 g). A sample (0.5 g) was purified on column chromatography (cellulose microcrystalline) obtaining **3b** analytically pure (0.3g): m.p. 250°C; IR (nujol): 3350, 1660, 1620 cm^{-1}; UV λ_{max}^{MeOH} 270nm (ε=18,500), 372 (16,000); NMR (DMSO-d_6): δ 2.8 (8H, m, $N(CH_3)_2$, C_{4a}-H, C_{5a}-H), 3.4 (2H, s, CH_2OH), 3.7 (1H, d, C_4-H), 6.8~7.7 (3H, m, aromatics), 9.0~9.7 (2H, d, $CONH_2$).

Anal. Calcd for $C_{22}H_{24}O_{10}N_2$: C, 55.46; H, 5.08; N, 5.88.
Found: C, 55.02; H, 4.92; N, 5.71.

6-Hydroxyanhydrooxytetracycline (**4**)

To a solution of **2b** (0.5 g, 0.9 mmoles) in MeOH - H_2O (1: 1, 15 ml), periodic acid (0.2 g, 0.9 mmoles) was added under stirring. The reaction was stirred for 3 hours, then poured in water, the pH adjusted to 6.5 and the compound extracted in *n*-BuOH (3×100 ml). The organic layer dried on Na_2SO_4 and evaporated *in vacuo*. The crude yellow-orange product (0.32 g, 80% yields) was crystallized from MeOH-Et_2O: m.p. 187~190°C; IR (nujol) 3500~3300, 1730, 1660, 1620 cm^{-1}; UV λ_{max}^{MeOH} 274 mm (ε=16,000), 471 (8,000).

Anal. Calcd for $C_{21}H_{18}O_9N_2$: C, 57.01; H, 4.1; N, 6.33.
Found: C, 57.18; H, 4.0; N, 6.28.

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