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# SYNTHESIS OF SOME NOVEL C-2 DERIVATIVES IN THE TETRACYCLINE SERIES

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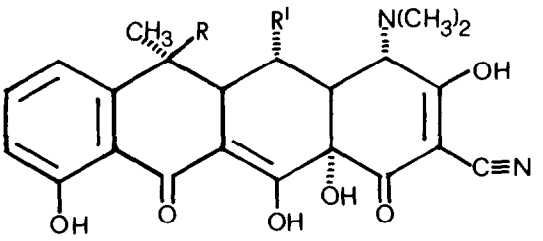
Summary: The 2-ethoxycarbonyl-2-decarboxamidodoxycycline 3 has been achieved starting from doxycyclinenitrile 1b. By reaction of doxycycline hydrochloride with $P_2S_5$ in dioxane it was obtained the 2-thiocarboxamido-2-decarboxamidodoxycycline 4 from which, with a Raney-Nickel reduction, it was possible to isolate the 2-methylamino-2-decarboxamidodoxycycline 5.

Although a very large number of tetracycline derivatives have been studied over the past years, a structure-activity relationship is not yet completely clear[1].

While the linear arrangement of the four rings and the two chromophoric keto-enol systems in ring A and rings BCD, constitute an important prerequisite, it seems that the hydrophobic part of the molecule ($C_5$-$C_9$) may be modified in various ways[2] in order to improve the antibiotic activity. At the C-2 position, it seems that the attached carbonyl is essential for the antibiotic activity: thus nitrile 1a[3] has no activity but 2-acetyl-2-decarboxamidotetracycline 2[4] (obtained by fermentation) and several derivatives substituted on the carboxamide nitrogen, posses some antibacterial activity.



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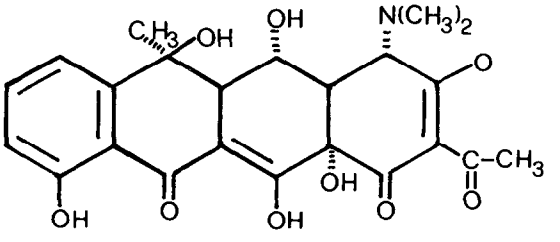
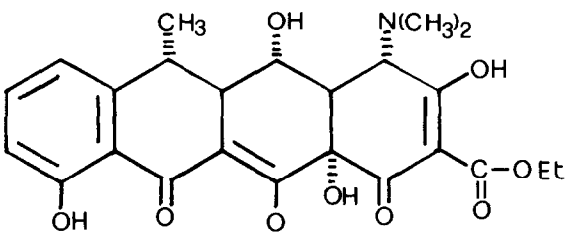
a, R = R' = OH
b, R = H, R' = OH



2

In order to reach a more clear structure-activity correlation, we are developing a research on new compounds in the tetracycline series and in the present communication we wish to report some C-2 doxycycline derivatives obtained during this work.

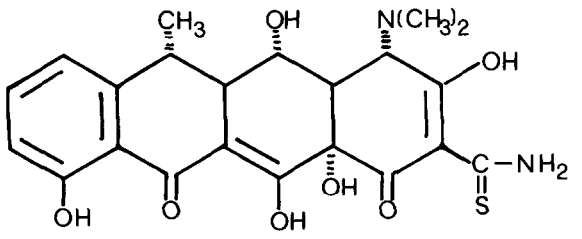
Treatment of the doxycyclinenitrile 1b [obtained from doxycycline hydrochloride with excess (2 eq.) of N,N-dicyclohexylcarbodiimide (DCC) in MeOH for 6 hr at room temperature: mp 226-228°C; ir (nujol) 2200, 1620 $cm^{-1}$; uv (MeOH) 270 ($\varepsilon$ =18,100) 360 nm($\varepsilon$ =13,000); $[\alpha]_D^{20°C}$= -74.01 (c=0.05% MeOH)[5]] with hydrogen chloride in EtOH at reflux for 30 hr, followed by concentration under vacuum and purification of the crude product by column chromatography (Silica gel treated with EDTA at pH= 7.4 and dried at 110°C, eluted with $CH_3COCH_3$/EtOAc/$H_2O$ 80/35/15 and then cellulose eluted with MeOH) gave the 2-ethoxycarbonyl-2-decarboxamido-doxycycline 3 in poor yield (18%): mp=188-190°C; ir (nujol) 1730, 1620 $cm^{-1}$; uv (MeOH) 265 ($\varepsilon$ =17,700), 360 nm ($\varepsilon$ =13,500); nmr[6]($CF_3COOH$): $\delta$ 1.1 (3H,d,$CH_3$-$C_6$), 1.6(3H,t,$CH_3$-$CH_2$-O), 2.7(6H,s,$N(CH_3)_2$),3.1-3.6(3H,m,$C_6H$,$C_{5a}H$,$C_{4a}H$),3.7-4.1 (3H,m,$C_4H$,$CH_2$),5.0(1H,d,$C_5H$),6.2-7.0(3H,m, aromatics); $[\alpha]_D^{20°C}$=+35,3 (c=0.02% MeOH)[5]



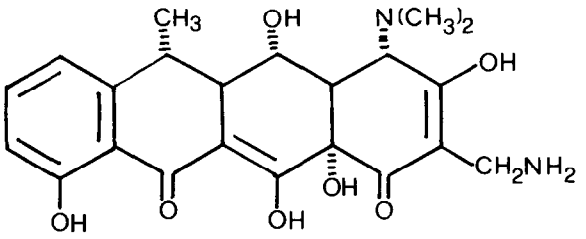

3

Moreover, reaction of doxycycline hydrocloride (5g, 10.4 mmoles) with $P_2S_5$ (2.87g, 12.5 mmoles) in dioxane (100 ml) for 18 hr at room temperature followed by purification of the crude product (obtained by diluting with $H_2O$ extracting in n-BuOH and concentration under vacuum) by column chromatography (silica gel treated as for 3 eluting with $CH_3COCH_3$/EtOAc/$H_2O$ 80/35/15) gave the 2-thiocarboxamide-2-decarboxamidodoxycycline 4 as a yellow matter (2.8g, yield, 58.3%) which was crystallized from MeOH:mp=222-225°C;ir(nujol)3340, 1620 $cm^{-1}$;uv(MeOH)270 ($\varepsilon$ =18,000)360nm( $\varepsilon$ =14,000);nmr[6]($CF_3COOH$) $\delta$ :1.1(3H,d,$CH_3C_6$), 2.7(6H,s,$N(CH_3)_2$), 3.1-3.6(3H,m,$C_6H$,$C_{5a}H$,$C_{4a}H$),3.9(1H,d,$C_4H$),4.8(1H,d,$C_5H$),6.2-7.0(3H,m,aromatics), 9.1(2H,s,$CSNH_2$); $[\alpha]_D^{20°C}$= -180.33(c=0.1% MeOH); S%found 6.62 calculated 6.93[5].

The structure of 4 was also confirmed by some chemical proofs: 1b does not react with $P_2S_5$, that is only the carboxamido group of doxycycline is involved in this reaction, and, moreover, 4 does not react, like doxycycline does, with DCC in MeOH to give the corresponding nitrile derivative.



4



5

Compound 4 gave a positive reaction with alkali plumbite according to[7]. The compound 4 was reduced with Raney-Nickel under hydrogen to give in high yields a compound, to which, by analytical data, was assigned the structure 5. A typical procedure for the synthesis of 5 was a follows[8]. A solution of 4 (2g, 4.3 mmoles) in EtOH was stirred under $H_2$ at room temperature with freshly prepared Raney-Nickel (10g) until disappearance of 4 (t.l.c. monitoring). Filtration, concentration under vacuum and crystallization of the crude product from MeOH-$Et_2O$ gave 5 (1.6g, yield, 86%). The product 5 was not very stable, but freshly prepared gave: mp= 212°C(dec); ir (nujol) 3340, 1620$cm^{-1}$; uv(MeOH) 265 ($\varepsilon$ =16,200), 360 nm ($\varepsilon$ =12,000); nmr(DMSO, $d_6$): disappearance of the band of $CSNH_2$; S%=0[5]. According nmr spectra and chemical proofs the asymmetric centers at $C_4$, $C_{4a}$, $C_5$, $C_{5a}$, $C_6$, $C_{12a}$ in 3, 4, 5 are the same as in doxycycline and in 1

All these compounds 3, 4, 5 were tested for microbiological activity (MIC) on agar plate against Staphilococcus aureus, Escherichia Coli, Klebsiella pneumoniae, Shigella flexneri and were pratically inactive in respect to the parent doxycycline.

According to our present results it seem therefore that the carboxamido group at $C_2$ position in doxycycline is very important for antibiotic activity.

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## REFERENCES AND NOTES

(1) W. Durckheimer, Ang. Chem. Int. Ed., 14, 721 (1975)

(2) R.K. Blackwood, A.R. English in "Structure-Activity Relationships among Semi-Synthetic Antibiotics" Ed. D. Perlman, Academic Press, N.Y. 1977, p 397 ss

(3) C.A. Stephens, U.S. Patent 3.028.409; C.A., 57, 9769g (1962)

(4) M.W.Miller and F.A. Hochstein, J. Org. Chem., 27, 2525 (1962)

(5) Elemental analysis gave satisfactory values.

(6) The 60 MHz nmr spectra were recorded on a Perkin Elmer R24 B spectrometer using TMS as internal standard.

(7) F. Feigl, "Spot test in Organic Analysis" 7th engl Ed, Elsevier, Amsterda 1966, p304

(8) Under same conditions, the doxycycline did not react and was recovered unchanged after a longer reaction-time.

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