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Free Radicals in Aqueous Alkaline Solutions of Tetracycline Derivatives

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Using the technique of electron spin resonance (ESR), it has been observed that solutions of several tetracycline derivatives in aqueous sodium hydroxide contain a relatively large concentration of free radicals. The ESR-spectra of these radicals have a characteristic appearance. The ESR-spectrum of the radicals present in a 0.02 M solution of 7-chlorotetracycline (aureomycin) hydrochloride in 1 N NaOH thus exhibited a doublet splitting with a hyperfine coupling constant of 4.9 gauss. Each one of the two lines was further split into a triplet with a coupling constant of about 0.5 gauss (Fig. 1).

Immediately after dissolving the substance, the radical concentration was found to increase slowly from zero up to a maximum value after about 2 h. An optimal resolution of the spectrum was obtained about 20 min after the substance had been dissolved. In this case the freshly prepared solution had been kept in an open vessel for 5 min before being filled into the sample cell. The resolution was found to increase with a decreasing microwave power applied

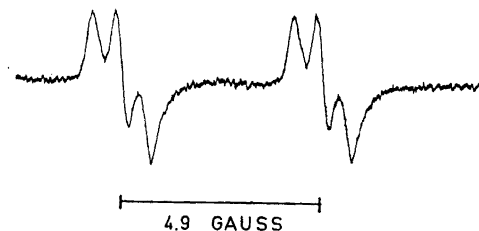


Fig. 1. ESR-spectrum of the free radicals formed from 7-chlorotetracycline hydrochloride dissolved in 1 N NaOH in H₂O.

to the sample, but was not improved by degassing of a solution in which radicals had already been formed.

On dissolving the substance in 2 N or 5 N NaOH, the formation of radicals was found to be more rapid than in 1 N NaOH, especially when the solution was kept in an open vessel. Primarily, the ESR-spectrum was identical with that obtained in 1 N NaOH (Fig. 1), but after some time a new line appeared which was located in the center between the original two lines. The center line gradually increased in magnitude and finally dominated the entire spectrum. No center line of this type could be observed in solutions of tetracycline derivatives when prepared from 1 N NaOH. When the experiments were performed with 0.1 N NaOH, there were no radicals to be observed.

ESR-spectra, identical with that obtained with 7-chlorotetracycline (Fig. 1), were also observed with tetracycline (achromycin), 5-hydroxytetracycline (terramycin) and 6-demethyl-7-chlorotetracycline (Ledermycin).

When 7-chlorotetracycline hydrochloride was dissolved in 1 N sodium hydroxide (NaOH or NaOD) prepared with D₂O, a new structure appeared in the center part of the spectrum between the original 2 × 3 lines. This structure seemed to be of a nature different from that observed with an aureomycin solution prepared with 2 N or 5 N NaOH in H₂O, and consisted of five lines, each one of which was further split into at least four lines. The magnitude of this central structure slowly increased and dominated the spectrum after about 1 h (Fig. 2). At the same time it was noted that the intensity of the original 2 × 3 line structure had been reduced.

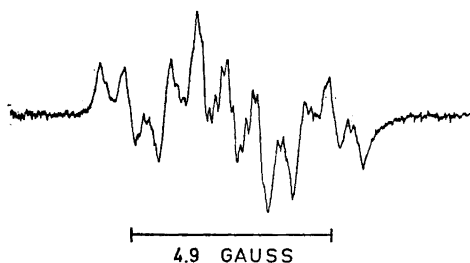


Fig. 2. ESR-spectrum of the free radicals formed from 7-chlorotetracycline hydrochloride dissolved in 1 N NaOD in D_2O . The spectrum was recorded about one hour after the substance was dissolved in the alkaline medium.

The doublet splitting of 4.9 gauss, observed in the radical spectra obtained with tetracycline derivatives dissolved in 1 N NaOH in H_2O (Fig. 1), is consistent with an interaction of the unpaired electron with one proton. It is suggested that the triplet splitting of the two main lines is due to an interaction with one ^{14}N nucleus as the intensity ratio of these lines seems to be close to 1:1:1. The appearance of the central five-line structure in a solution prepared from 1 N sodium hydroxide in D_2O is suggested as originating from an exchange of the proton which gives rise to the doublet splitting, for deuterium. Consequently, the central five-line structure might be interpreted as due to a combined interaction of one ^{14}N and one deuterium nucleus (nuclear spin of deuterium = 1) assuming an approximately equal splitting for these two nuclei. Although a hyperfine structure due to an interaction with deuterium nuclei seems to have been observed rather seldom, the resolution of such structures has been described for some radical systems. The ratio between the hyperfine splittings due to a proton and a deuterium nucleus on identical positions in radicals, *i.e.* $a_H:a_D$, has been found to be close to 6.5.^{1,2} The hypothesis of a resolved structure due to an interaction with deuterium in the spectra concerned in this case is partly supported by an observed ratio of 7.2 between the doublet splitting and the splittings of the central five-line structure.

No secondary splittings corresponding to those observed in the central five-line structure (Fig. 2) could be detected in the spectra of the radicals present in the solu-

tions prepared with H_2O . The cause of this discrepancy is not understood. It may possibly be due to an exchange of other protons in the radicals, by this means effecting a higher resolution of structures otherwise obscured by overlapping absorptions.

On the basis of the ESR-spectra recorded and the somewhat speculative interpretation of the above-mentioned spectra, it seems to be difficult to express any definite opinion as to the chemical structure of the radicals obtained. In the first place it is not clear whether the radicals are derived from the intact tetracycline substances by a redox-reaction, or whether they are formed from some of the degradation products known to be produced from tetracyclines in alkaline media.^{3,4} The last alternative seems to be most probable, since iso-aureomycin and aureomycinic acid (Fig. 3), which are produced by alkaline

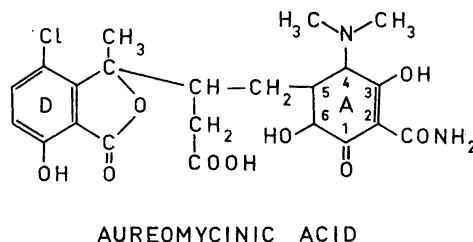


Fig. 3.

degradation of aureomycin (Fig. 4), were both found to give rise to radicals when dissolved in 1 N NaOH, exhibiting ESR-spectra almost identical with those obtained from aureomycin (Fig. 1). It could

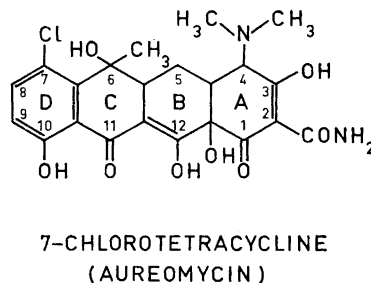


Fig. 4.

not be decided from these experiments, however, whether iso-aureomycin or aureomycinic acid, or some other degradation product of aureomycin, constitutes the structural basis of the observed radicals. Consequently, it would appear impossible to make any statement regarding the structural position of the proton which is suggested as giving rise to the doublet splitting in the spectrum shown in Fig. 1. If the triplet splitting of the two main lines present in the spectrum obtained in alkaline H₂O (Fig. 1) is really due to an interaction with one ¹⁴N nucleus, then the radicals are very probably related to a structure derived from ring A of the tetracycline molecule. This statement is consistent with the formation of the characteristic radical spectrum with iso-aureomycin and aureomycinic acid.

The presence of hydroxy and oxo groups on the ring system of tetracycline and the degradation products concerned, especially on ring A, infers that the free radicals may be associated with the formation of a semiquinone anion by oxidation with molecular oxygen.

The ESR-spectra were obtained with a Varian 100 kc spectrometer.

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Die Synthese von Trichlormethylarylsulfiden aus Trichlormethansulfenylchlorid und reaktiven Aromaten *

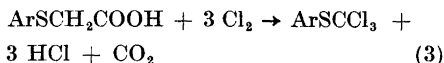
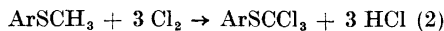
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Die Kernsubstituierung reaktiver Aromaten mit Trichlormethansulfenylchlorid (1)



stellt eine wertvolle Ergänzung der bekannteren Chlorierung von Methylarylsulfiden ¹ (2) bzw. von Arylmerkaptoessigsäuren ² (3) dar.



Argyle und Dyson ³ untersuchten 1937 die bereits 1886 von Rathke ⁴ beobachtete Bildung von Kristallviolett aus N,N-Dimethylanilin und CCl₃SCl näher und fanden, dass die Reaktion in erster Stufe nach (1) verläuft (4); wobei sich die Bildung von Kristallviolett jedoch nicht ganz unterdrücken lässt.

Die vorliegende Arbeit hatte zum Ziel, den Gültigkeitsbereich dieser Reaktion abzustecken sowie den Einfluss von Friedel-Crafts-Katalysatoren zu untersuchen.

Die Versuche mit Friedel-Crafts-Katalysatoren verschiedener Aktivität (z. B. AlCl₃, BF₃, SnCl₄) zeigten, dass die milderen Katalysatoren keinen Effekt haben, während Lewis-Säuren mit kräftiger Katalysatorwirkung die bekannte Reduktion des CCl₃SCl (im Gegenwart von Wasserstoffdonatoren) zu Thiophosgen ⁵ in den Vordergrund treten lassen.

Zwischen Phenolen (z. B. Phenol und Resorzin) und CCl₃SCl liess sich nur ein teilweiser Umsatz erzielen, der jedoch nur schlecht definierte tiefgefärbte polymere Produkte lieferte. Bei der Einwirkung von CCl₃SCl auf Anisol und Resorzindimethyläther liess sich eine (vermutlich radikalische) langsame Chlorierungsreaktion (un-

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