

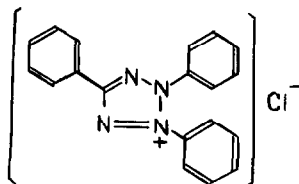
ESR SPECTRUM OF 2,3,5-TRIPHENYL TETRAZOLIUM

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2,3,5-TRIPHENYL tetrazolium chloride has been widely used as a staining reagent for living mate-



2,3,5-triphenyl tetrazolium  
chloride

rials to test biochemical  
redox activity.<sup>1</sup>

Using the well-known  
reducing method with sil-  
ver amalgam in vacuo, 2, 3,

5-triphenyl tetrazolium  
chloride was reduced in 1,  
2-dimethoxy ethane (DME).

The solution turned to red gradually and showed an ESR  
spectrum.

It was a peculiar feature of this spectrum that the  
intensity increased day after day accompanying strong col-  
oring of the solution. This means that reduction of 2, 3,  
5-triphenyl tetrazolium chloride with silver amalgam pro-  
ceeds very slowly and the radical thus produced is fair-  
ly stable.

Fig. 1 is the ESR spectrum due to the radical by  
reduction of 2,3,5-triphenyl tetrazolium chloride with  
silver amalgam. The g-value of this radical is 2.0037  
 $\pm$  0.00005 and the integrated intensity ratios of each  
line agree well with those of the theoretical spectrum,

<sup>1</sup>R. Kuhn and D. Jerchel, Ber. 74, 941, 949 (1941).

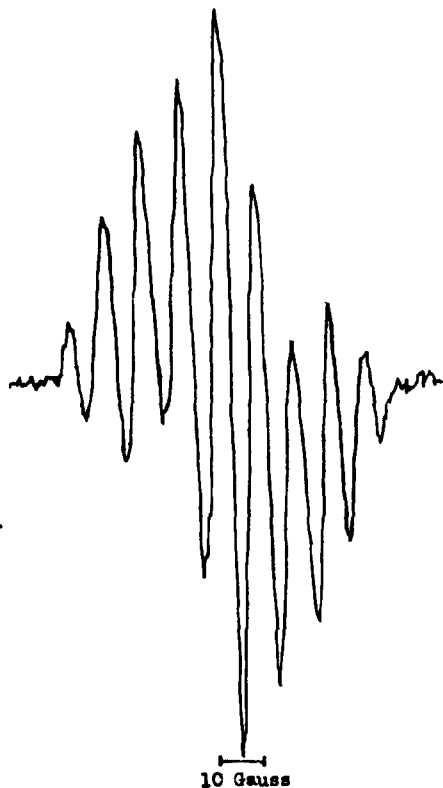


Fig.1. ESR spectrum of the radical  
produced by reduction of  
tetrazolium chloride with sil-  
ver amalgam in DME.

provided we analyse this spectrum as  $A_N = 6.4$  Gauss, assuming four  $^{14}\text{N}$  nuclei are equivalent. From these results, it was concluded that this spectrum should be identified as that of 2,3,5-triphenyl tetrazolium radical.

If reduced in tetrahydrofuran (THF), the same result as above was obtained but decay of the produced radical was rapid. Using zinc amalgam or zinc dust in THF, the same color solution was obtained but was too unstable to observe an ESR spectrum.

In ethyl alcohol we could obtain the red solution but not any ESR spectrum.

The above mentioned results were also obtained in the case of the samples prepared in the open air.

Further investigation is now going on to obtain more resolution of the ESR spectra due to the hyperfine interaction of the protons of the phenyl rings.