

## On the Alkaline Deacylation of 1-Acyl-2-thiohydantoin

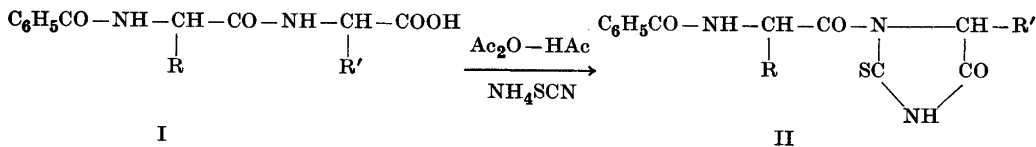
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The recent interest in the stepwise degradation of peptides<sup>1-3</sup> aroused our interest in the method of Schlaack and Kumpf<sup>4</sup>, involving the facile formation of 1-acylated-2-thiohydantoin (II) on treating suitable N-benzoylated peptides (I) with ammonium thiocyanate in mixtures of acetic anhydride and acetic acid. This reaction represents an extension of the Komatsu-Johnson-procedure<sup>5,6</sup> for converting N-acylated  $\alpha$ -amino acids into 2-thiohydantoin.

different and quite characteristic, the former having two peaks located at ca. 235  $m\mu$  and 275–280  $m\mu$  with high extinctions ( $\epsilon_{\text{mol}}$ : 15–20000), while the thiohydantoin shows maximum absorption at ca. 225  $m\mu$  and 260  $m\mu$  with large differences in extinction ( $\epsilon_{\text{mol}}$ : 10000 and 17000, respectively). These measurements, made in 0.001 *N* hydrochloric acid solution (Fig. 1), are in good agreement with the results obtained in alcoholic solution during the penicillin programme<sup>7</sup>.

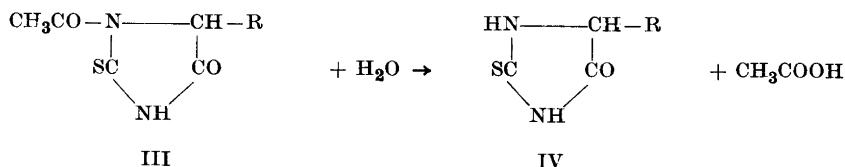
Samples of different 1-acetyl-2-thiohydantoin (III, R = *iso*-C<sub>4</sub>H<sub>9</sub>, C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub> and H) were dissolved in 2 equivalents of 0.1 *N* sodium hydroxide at room temperature and immediately upon dissolution an aliquot was withdrawn, diluted and made 0.001 *N* in hydrochloric acid. Much to our



While Johnson and coworkers consistently employed a fairly vigorous treatment with strong acid in order to detach the acyl-group located in 1-position of the thiohydantoin, Schlaack and Kumpf (*l.c.*) demonstrated the hydrolytic fission to take place with 1 *N* sodium hydroxide at room temperature.

With a view to applying the method on higher peptides, still milder conditions were desired and found through spectrophotometric studies. The UV-spectra of 1-acylated- and unsubstituted 2-thiohydantoin, respectively, appear to be very

surprise, the absorption data disclosed that the fission had proceeded practically to hundred per cent in less than five minutes. Parallel experiments in 0.01 *N* sodium hydroxide revealed that even this extraordinary mild treatment achieves the complete fission in about 30 minutes. Again, the benzoylglycyl-residue in 1-hippuryl-2-thiohydantoin (II, R = R' = H) was detached completely in less than five minutes upon treatment with 0.1 *N* sodium hydroxide as seen from absorption measurements. These findings, adopted to a synthetical scale, resulted in a quantita-



R	M.p.	Nitrogen %		Sulphur %	
		Calc.	Found	Calc.	Found
(CH <sub>3</sub> ) <sub>2</sub> CH	114°	13.99	13.87	16.01	15.90
CH <sub>3</sub> (C <sub>2</sub> H <sub>5</sub> )CH	153–158° *	13.08	13.25	14.96	14.90
CH <sub>3</sub> SCH <sub>2</sub> CH <sub>2</sub>	105–106°	12.06	12.54	27.59	27.84

\* The wide melting range indicates a mixture of stereoisomides.

tive yield of 2-thiohydantoin (IV, R = H) after dissolution of 1-acetyl-2-thiohydantoin, (III, R = H) in two equivalents of 0.1 *N* sodium hydroxyde, followed by immediate acidification and extraction with *n*-butanol.

An interesting observation was made when a freshly prepared solution of 1-benzoyl-2-thiohydantoin in water was followed spectrophotometrically. The starting curve (Fig. 2), which is reminiscent of the

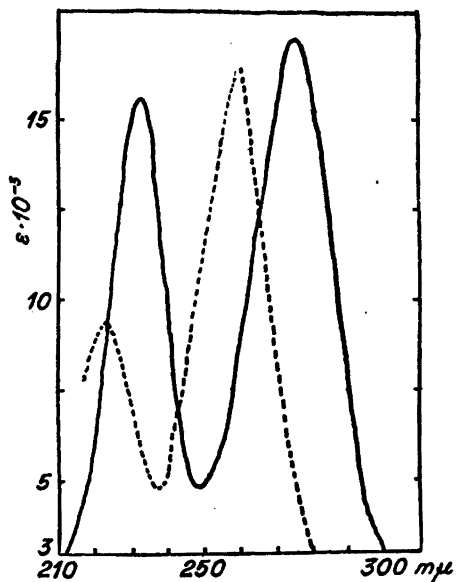


Fig. 1. Ultraviolet absorption spectra in 0.001 *N* HCl of ——— 1-acetyl-2-thiohydantoin and - - - - 2-thiohydantoin.

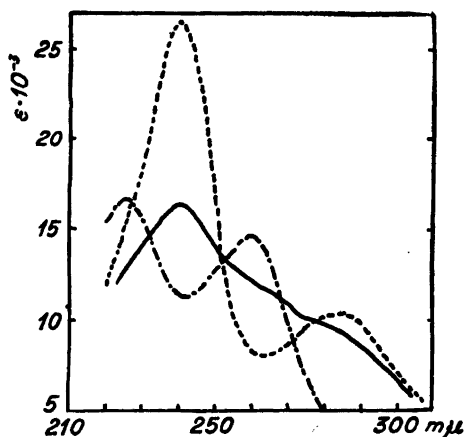
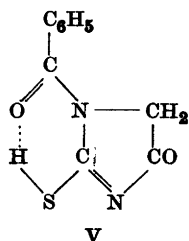


Fig. 2. Ultraviolet absorption spectra of 1-benzoyl-2-thiohydantoin in: - - - - 95% EtOH; ——— freshly prepared aqueous solution; - . . . . water after standing for 18 hours.

absorption data in ethanol, changes quite rapidly into a final curve, practically identical with the absorption data obtained in dilute acid solution and not differing principally from the curve in dilute alkali. As an explanation for this behaviour we tentatively suggest the presence in the neutral alcoholic solution of hydrogen-bonded molecules such as (V), whereas acid or alkali may break up the labile bond under simultaneous displacement of the absorption peak by about 15  $m\mu$  towards shorter wave lengths.



During the present work the following new DL-1-acetyl-2-thiohydantoin (III) were prepared from the appropriate  $\alpha$ -amino acids according to the usual procedure<sup>6</sup>.

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## Studies on Lignin. XII. \* Alkaline Desulphonation of Radioactive Lignin Sulphonic Acid

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When lignin sulphonic acids are heated with aqueous alkali, they gradually undergo desulphonation<sup>1,2</sup>, with the formation of sulphite. Under suitable

conditions this process can be effected stepwise<sup>3</sup>, and it was considered that a detailed study of the reaction might throw much light on the problem of the nature of the sulphonatable groups in lignin.

This preliminary communication describes some desulphonation experiments with a spruce "Kullgren acid" (S = 5.5 %, Ba/S = 0.50, S/OCH<sub>3</sub> = 0.49) which was further sulphonated with sulphite containing S<sup>35</sup>, and then analyzed as follows: S = 6.8 %, Ba/S = 0.53, S/OCH<sub>3</sub> = 0.68. The data obtained during desulphonation are listed in Table 1.

Table 1. Alkaline desulphonation of lignin sulphonic acid.

Time of alkali treatment minutes	Sulphur removed as SO <sub>2</sub> (c), % of total	Specific S <sup>35</sup> activity (relative values)
15	26.1	100 ± 0.5
30	30.2	97.7 ± 0.9
50	32.0	95.0 ± 0.7
110	36.7	91.3 ± 0.7
170	39.9	89.0 ± 0.4

The figures of the last column are averages of 4 measurements.

The statistical error is 0.4–0.5 %.

Initially, sulphite is formed rapidly, but subsequently much more slowly as is evident from Fig. 1, in which ln (100-c) is plotted against time.

The graph shows clearly that the desulphonation involves two reactions, one relatively fast and one relatively slow. In the rapid reaction about 30 % of the total sulphur is liberated. (This figure, obtained by extrapolation, changes only little if it be assumed that only e.g. 75 % of the total sulphur may be liberated.)

The specific activity of the liberated SO<sub>2</sub> decreases regularly with time, showing that the sulphur introduced in the second phase of the sulphonation is most readily

\* Part XI. *Svensk Kem. Tid.* **64** (1952) 18.