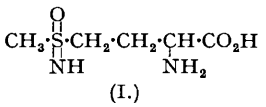
381. *Toxic Factor from Agenized Proteins : Infra-red Measurements.*

By L. N. Short and H. W. Thompson.

Infra-red spectra of the toxic factor from agenized proteins previously examined by other workers, and of compounds of related chemical structure, have been measured. The results confirm the suggestion that the toxic agent is the sulphoximine from L-methionine.

Since the discovery by Mellanby (*Brit. Med. J.*, 1946, II, 885; 1947, II, 288) that flour which has been treated with "Agene" (nitrogen trichloride) produces hysteria in dogs, attempts have been made to isolate the toxic factor involved. In a series of communications Bentley, McDermott, Pace, Whitehead, and Moran (*Nature*, 1949, **163**, 675; 1949, **164**, 438; 1950, **165**, 150) have described the separation of a compound which appeared to be responsible for the effect. On the basis of analysis and chemical reactivity, they suggested that it is a derivative of methionine having the structure (I), and have called this a sulphoximine.



(I.)

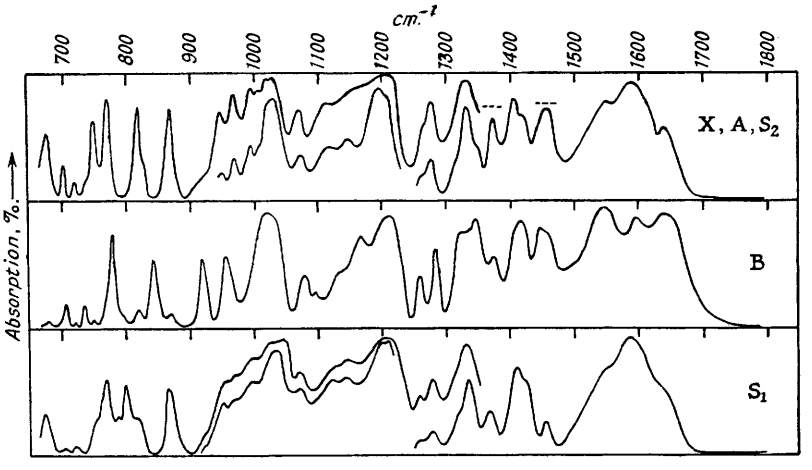
It was suggested that infra-red spectra might provide additional or confirmatory evidence, and we have therefore measured the spectra of the toxic factor and of a series of compounds, of structure related to (I), which were kindly provided for us by the above workers. The spectra were recorded with a Perkin–Elmer 12C instrument, using prisms of rock salt and lithium fluoride. The substances were examined as solid powders in paraffin and "perfluorokerosene."

We have measured the spectra of two samples of the toxic factor (X), and since they showed important differences this requires some explanation. Bentley, McDermott, Pace, Whitehead, and Moran found that the factor could be separated by means of the picrates and chromatographically into two parts A and B, which although apparently chemically identical, differed in toxicity. They suggested that A and B might be diastereoisomers. The first sample of the factor measured had an infra-red spectrum very similar to that of B, but also showing definite signs of bands of A, suggesting that it may have been an incompletely fractionated sample. The second specimen of the toxic factor had a spectrum identical with that of A.

The spectrum between 5 and 15 μ. of the second sample is shown in Fig. 1. It shows bands in the region 1300—1500, near 1600, and near 2900 cm.$^{-1}$ (not shown) which are compatible with its being some kind of amino-acid, although taken alone this is not very characteristic. It may be noted, however, that it has a band near 2120 cm.$^{-1}$ which is common to most simple amino-acids (cf. Nicholson, Short, and Thompson, *Discuss. Faraday Soc.*, 1950, **9**, 222). Fig. 2 shows the spectra of DL-methionine, DL-methionine sulphoxide, and DL-methionine sulphone. The similarity between the spectra is perhaps less close than might have been expected

FIG. 1.

Spectra of solids in Nujol.



for such closely related structures. Fig. 2 also includes the spectra of dimethyl sulphoximine, synthesized by Bentley *et al.* (*Nature*, 1950, **165**, 735; *J.*, 1950, 2081). Spectra are also included for *S*-methylcysteine and its sulphoxide and sulphoximine, the lower homologues of the methionine series.

The most striking feature of the spectrum of X is the occurrence of two strong bands near 1020 cm.$^{-1}$ and 1210 cm.$^{-1}$. Characteristic bands of the sulphoxide and sulphone groups have already been discussed by Barnard, Fabian, and Koch (*J.*, 1949, 2442) and by Schreiber (*Analyt. Chem.*, 1949, **21**, 1168). Sulphoxides give a band near 1035 cm.$^{-1}$, while sulphones have a pair at 1150 and 1290 cm.$^{-1}$. Bands at the appropriate frequencies occur as expected with methionine sulphoxide and sulphone, and *S*-methylcysteine sulphoxide. If X had the sulphoximine structure we might expect a pair of intense bands due respectively to the S=O and S=N bonds, as is indeed found. We can in fact imagine that in a sulphone each S=O link would in itself give rise to a frequency around 1200 cm.$^{-1}$, the contiguous pair splitting into the two observed bands at 1150 and 1290 cm.$^{-1}$. Thus we can reasonably attribute the band found with X at 1210 cm.$^{-1}$ to the S=O linkage, and then assign the band near 1020 cm.$^{-1}$ to the S=N bond. Unfortunately we have no other evidence about the relative strength of the S=O and S=N linkages which might suggest relative values for the vibration frequencies.

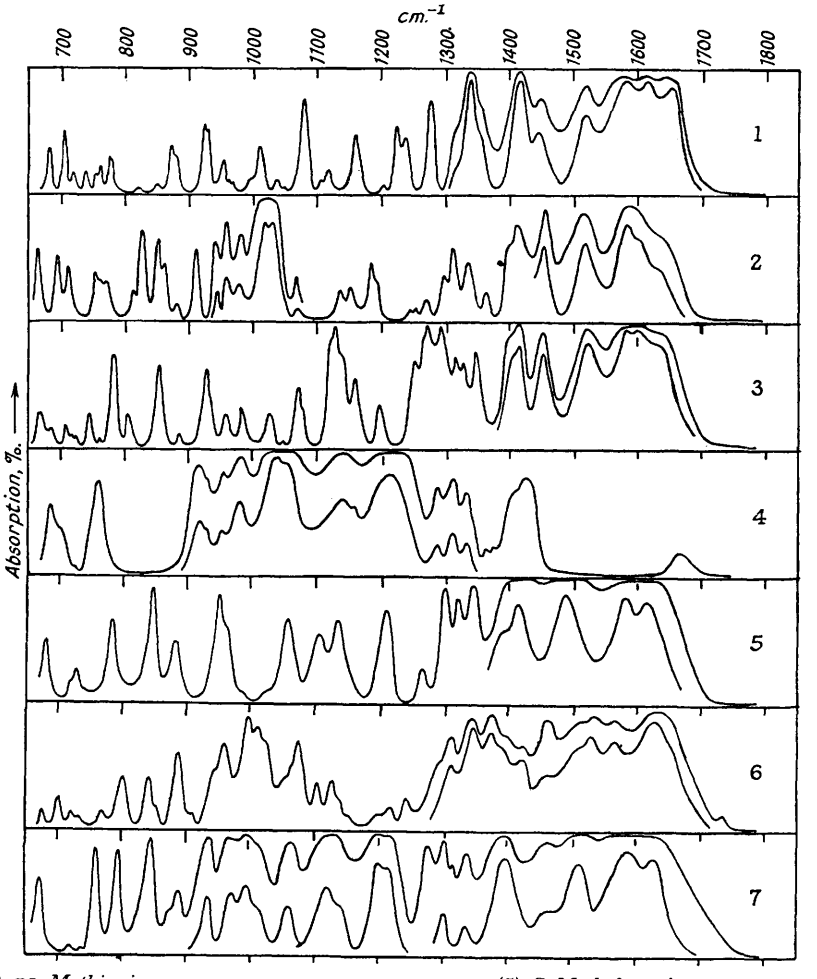
The synthetic sulphoximines from dimethyl sulphoxide and *S*-methylcysteine also have strong bands in the region of 1020 and 1210 cm.$^{-1}$.

Sulphoximines have also been synthesised from L-methionine and DL-methionine, these being labelled S_2 and S_1 respectively in Fig. 1. The spectrum of S_2 was identical with that of A

and of the second sample of X. This can be taken as perfect confirmation of structural identity, particularly in the light of the highly characteristic nature of the spectra already mentioned. The spectrum of S_1 differed from that of S_2 much as might be expected of an enantiomorph and a racemate, but it may also be noted that it differed also from the spectrum of B. The exact nature of B is therefore undecided, although the differences may be connected with stereo-isomerism about the sulphur atom taken together with such isomerism about the α-carbon atom.

FIG. 2.

Spectra of solids in Nujol.



(1) DL-*Methionine.*
(2) DL-*Methionine sulphoxide.*
(3) DL-*Methionine sulphone.*
(4) *Dimethyl sulphoximine.*
(5) S-*Methylcysteine.*
(6) S-*Methylcysteine sulphoxide.*
(7) S-*Methylcysteine sulphoximine.*

There is one alternative interpretation of the structure of X, namely that it is really an " oxime " of the type $>S{:}N{\cdot}OH$. There is good chemical evidence against this, and the spectrum also appears to exclude it. The " oxime " structure might be expected to show stretching and bending modes of the O–H bond near 3 and 10 μ., the exact positions being dependent on hydrogen-bonding relationships. There are indeed bands near these wave-lengths, but that near 3200 cm.$^{-1}$ could be equally explained by the $=NH$ group and we have already given a more reasonable assignment to the band at 1020 cm.$^{-1}$. Moreover, measurement of the spectra of simple oximes gave no strong bands of this kind near 1000 and 3200 cm.$^{-1}$. A further point is that partial deuteration of S_2, though introducing some small spectral changes

did not appreciably affect either of the bands near 1020 and 1210 cm.$^{-1}$. The band near 3200 cm.$^{-1}$ was displaced by deuteration to lower frequencies but this could of course equally well occur with an N–H or O–H bond.

All the above results therefore confirm the sulphoximine structure proposed for the toxic factor. Some of the minor spectral changes found in different compounds of related structure, especially those near 6 μ., might be caused by small variation in the degrees of hydrogen-bridge formation between S=O, NH, and the NH or C=O linkages in the amino-acid part of the skeleton caused by differences in the folding of the chain. Unfortunately we have no means of examining this further.

We are grateful to the workers at the Research Association of British Flour Millers for drawing our attention to this problem, and to the Australian National University for a Scholarship to one of us.

PHYSICAL CHEMISTRY LABORATORY, OXFORD UNIVERSITY. [*Received, January 27th,* 1951.]