chains also appeared to conform to our expectations.

At room temperature the stable form of the crystals turned out to be monoclinic (space group $P2_1m$) with two molecules in the unit cell. The parameters are:

$$a = 4.43 \text{ \AA}; b = 6.72 \text{ \AA}; c = 7.65 \text{ \AA}; \beta = 93.9^\circ$$

It appeared probable that the chains in this modification would turn out to run parallel to the [101] direction, the period of which is 8.58 Å. The correctness of this assumption has been verified by the crystal structure determination which followed. From intensity data based on zero layer Weissenberg diagrams with rotation about [010], [100], and [001], Patterson and Fourier maps were computed followed by least squares refinements. The atomic parameters listed in Table 1 were obtained. This table also contains the interatomic distances within the chains.

The final R values were: $R_{hol} = 0.05$; $R_{hko} = 0.05$; $R_{okl} = 0.08$. The B values arrived at show that the vibration amplitudes of iodine are larger perpendicular to the chains than along the chains.

In the low temperature form the distance between neighbouring chains (which are all running in the same direction) is 3.93 Å. In the monoclinic form stable at room temperature the chains are situated in the planes $y = \frac{1}{4}$ and $y = \frac{3}{4}$, the chain direction being the same within a particular layer but changing 180° from layer to layer. The situation is visualized schematically in Fig. 1 a (low temperature form) and 1 b (monoclinic form). It is worth mentioning that the distances between neighbouring chains in the monoclinic form, 3.91, 3.94, and 3.97 Å has a mean value 3.94 closely corresponding to that observed in the low temperature modification (3.93 Å) and that the packing is very much the same in both cases. Corresponding observations have been made in the case of the cyanogen halides². In the orthorhombic cyanogen bromide, however, the two different values found for the distances between neighbouring chains differ more than in the monoclinic iodo-cyano-acetylene.

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Note on Ascorbigen as a Source of Vitamin C

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Recently Kiesvaara and Virtanen (K. and V.) published a paper in this journal¹ (see also Ref.²), in which they state, in accordance with older work by Sumtsov³, that ascorbigen possesses "vitamin C effect". We have also studied the question of the antiscorbutic activity of ascorbigen but arrived at essentially the opposite conclusions^{4,5}.

K. and V. found² that a 15 mg dose of ascorbic acid per guinea pig of 250–300 g body weight per day had the same effect on the weight increase of animals fed on a scorbutogenic diet as 26 mg of their preparation of synthetic ascorbigen.

On the other hand we have found⁵ that a 10 mg dose of ascorbigen per guinea pig of 120–150 g body weight per day (corresponding to 20 mg of ascorbigen for older guinea pigs as used by K. and V.) did not show any curative effect on animals kept on a scorbutogenic diet, whereas even 0.5 mg of ascorbic acid had a full curative effect. The same effect was obtained by a 1 mg dose of hydrolysed ascorbigen⁴. Scurvy was diagnosed in our experiments on younger animals, which are more sensitive to vitamin C, not only by the weight decrease of the animals or their external appearance, but also by the more sensitive and reliable odontoblast test⁶.

The following facts emerge in a comparison of the two sets of results: The curative dose of ascorbic acid per animal (250–300 g) per day is 1 mg. However, in their control experiment K. and V. administered 15 times this amount to scorbutic animals, thus invalidating their results for quantitative purposes. Only a qualitative conclusion can be drawn from their experiments, *i.e.* that a very high dose of their preparation can, under their experimental conditions, prevent the experimentally induced scurvy. However, even the results of Feldheim⁴ (which are

unfortunately not quoted by K. and V.) give a clearer picture in spite of the fact that owing to lack of ascorbigen he could compare only the minimum curative dose of ascorbic acid (0.5 mg per animal of 125–130 g daily) with the same amount of bound ascorbic acid, *i.e.* with 1 mg of ascorbigen. This shows that at this critical dose ascorbigen is completely inactive, or, in other words, that its activity must be less than half that of L-ascorbic acid. We therefore fed our animals with large doses of ascorbigen⁵ in order to establish, perhaps even quantitatively, the activity of ascorbigen relative to L-ascorbic acid. However, this problem was only partially solved. We concluded from our experiments that free ascorbic acid is over 20 times more active than ascorbigen. We have therefore critically reexamined both sets of experimental conditions^{1,5} and have arrived at the following conclusions.

In their diet, as described by K. and V., older, less sensitive guinea pigs were given daily 0.3 mg of ascorbic acid contained in the dried grass, and another 0.13 mg contained in their preparation of ascorbigen. This is a total of 0.43 mg, which represents almost one-half (43 %) of the minimum curative dose. From the description of the preparation of ascorbigen by the Finnish authors it follows that the reaction mixture (10 g of indole, 30 g of ascorbic acid, 5.2 g of formaldehyde in 400 ml of a 0.05 M citrate buffer were mixed and heated at 54° for 7–8 h) was merely filtered and the filtrate, which necessarily contained large amounts of ascorbic acid, and possibly some dehydroascorbic acid, was worked up in such a way that appreciable amounts of these must have been extracted, as we have demonstrated in our own experiments⁵. The ascorbic acid and other impurities were removed from our extracts by chromatography on a polyamide column and by fractional precipitation. K. and V., on the other hand, only concentrated and dried their extract. They state, that their material "according to paper chromatograms and the UV-spectrum was pure ascorbigen". According to our experiences neither paper chromatography nor UV-spectrophotometry are sufficiently reliable criteria for the purity of ascorbigen. The ascorbigen of K. and V. must have contained, as was already pointed out, a certain amount of ascorbic acid that passed from the aqueous phase into the ethyl acetate layer during the extraction. In order

to verify this possibility we repeated their experiments. We reproduced their method of ascorbigen synthesis¹ (see above) on one-tenth of their scale, and obtained one-third of the yield, quoted by them. The UV-spectra and paper chromatograms clearly showed the presence of ascorbic acid in the preparation. According to 2,6-dichlorophenol indophenol titration the ascorbigen sample thus obtained¹ contained approximately 5 % of free ascorbic acid, *i.e.* 10 times the amount quoted by the Finnish authors. If an aqueous solution of this sample was left to stand the ascorbic acid content increased and was doubled after 3 h. This rapid increase of ascorbic acid cannot be explained by the hydrolysis of ascorbigen since this is extremely slow; in neutral medium according to Ettlinger⁷ it occurs to the extent of 1 % per hour at most, and according to Procházka and Jindra⁸ hardly at all. Hence the concentrate must have contained ascorbic acid combined in a form more labile than ascorbigen. It is known⁹ that the reducing power of ascorbic acid is rapidly diminished at certain pH values in the presence of formaldehyde. We therefore carried out a control experiment, again using the above mentioned procedure of the Finnish authors, but leaving out the indole from the reaction mixture. The resulting product, whose weight was only one-half of that obtained in the presence of indole, and which could not contain any ascorbigen, contained approximately 2.3 % of free ascorbic acid and no dehydroascorbic acid. When an aqueous solution of this extract was left to stand, the titre of ascorbic acid increased steadily until it reached a value eight times that of the original solution, *i.e.* the ascorbic acid content (free + combined with formaldehyde) was 18.4 %. In a subsequent experiment we showed that under the conditions used (3 g of ascorbic acid, 0.52 g of formaldehyde, in 40 ml of 0.05 M citrate buffer) ascorbic acid reacts very rapidly in the cold (80 % after 1.5 h), and that the complex can be extracted with ethyl acetate, although much less readily than ascorbigen. Experiments with the extracted complex showed that it is more stable in 1 % sulphuric acid than in distilled water. It is quite stable in organic solvents.

Thus it is probable that the preparation of K. and V. contained ascorbic acid, both free and combined with formaldehyde, in sufficient amount to produce a curative effect on experimental scurvy when a 26 mg dose per animal and per day was

administered. Such a dose of a preparation made by us according to their prescription contained 1.3 mg of free ascorbic acid and 1.3 mg combined with formaldehyde. Under the conditions and diet used by K. and V. 0.7 mg or less of ascorbic acid would have sufficed to cure the scurvy.

The possibility that the dose tested by K. and V. represents the threshold dose under their experimental conditions should be verified, as should the possibility that their animals were more sensitive, or able to hydrolyze ascorbigen *in vivo*.

Our theory regarding the inactivity, or rather the very low activity of ascorbigen is also supported by our findings that ascorbigen is not hydrolysed in the stomach and that it rapidly passes into the blood stream, where, as a result of a relatively low hydrogen ion concentration (pH 7.4), it is quickly converted into a material (substance C¹⁰) which does not liberate ascorbic acid any more, and that most of it is excreted in the urine either unchanged or in the form of substance C.

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