determine the product composition. In the enone case both radical models predict the same products, but the high stereospecificity of the reaction[6] seems to imply that bond formation and thus the free radical density is the most important factor.

It seems clear that the photo cyclo addition of α,β-unsaturated carbonyl compounds to olefins has partial electrophilic character. However, the approximative nature of the present calculations and the lack of experimental data make the conclusions uncertain. More refined calculations and more experiments are under way in an attempt to clarify the situation.

Experimental. The parameters used in the Hückel MO calculations were essentially those recommended by Streitwieser:[8] $\alpha_{=O}=\alpha+\beta$, $\beta_{C=O}=\beta$, $\alpha_{C-CH_3}=\alpha-0.5\beta$ ("inductive model"), $\alpha_{CH_3}=\alpha+2\beta$, $\beta_{C-CH_3}=0.7\beta$ ("hetero atom model"). It was assumed that the oxygen n-electron could be incorporated in the π^*-orbital without corrections. However, since the electron affinity of the carbonyl oxygen should be considerably higher in the $n\rightarrow\pi^*$ excited state than in the ground state, calculations were also carried out using $\alpha_{=O}=\alpha+2.5\beta$. The results of the calculations are presented as electron density in the first excited orbital (Table 2) and relative charge (Table 1). The charge on the oxygen is corrected for the loss of one n-electron.

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Adaptation of the 2,4-Dinitrophenylhydrazine Method for Determination of Ascorbic Acid after Separation by Column Chromatography

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The colorimetric procedure, based on the reagent 2,4-dinitrophenylhydrazine (2,4-DNPH), developed by Roe *et al.*,[1-4] is the most specific and most commonly used method for the determination of ascorbic acid (AA) in biological materials. In this procedure, a trichloroacetic acid (blood, urine) or metaphosphoric acid (plant materials) filtrate is shaken with active charcoal and filtered. The charcoal clarifies the solution and oxidizes the AA to dehydroascorbic acid (DHA). The filtrate is incubated with 2,4-DNPH for 3 h at 37°C in the presence of thiourea. Sulfuric acid, 16 M, is then added to the tubes in an ice bath. After 30 min absorption is measured at 520 nm in a colorimeter.

In its original version, the method is rather laborious and time-consuming. The present paper describes a simplified version which permits the convenient processing of a large number of samples. It was developed for use with a column chromatographic procedure for the assay of trace amounts of vitamin C in dried feed stuffs.[5] The adaptation of the 2,4-DNPH method for large scale work involves the use of 2,6-dichlorophenolindophenol (2,6-DCPI) as oxidant. Incubation is carried out at an elevated temperature for 1 h. Full use is

made of automatic transfer and pipetting devices.

Reagents. a) *2,6-DCPI reagent.* 0.2 % (w/v) 2,6-DCPI in boiled, redistilled water. The solution is filtered and kept in a brown bottle in the refrigerator. To be freshly prepared weekly. b) *2,4-DNPH reagent.* 4.5 M sulfuric acid containing 2 % (w/v) 2,4-DNPH and 4 % (w/v) thiourea. The reagent is kept in the refrigerator and filtered on a glass filter before use. The adequacy of the thiourea content is checked by adding the reagent dropwise to a few ml of 1 % $HgCl_2$ in a test tube, which should produce a white precipitate. c) 16 M sulfuric acid.

Standard solutions. For 1 cm cuvettes: 2, 5, 10, and 20 μg of AA per ml in 2.5 M acetic acid (HAc) containing 0.1 % 2-mercaptoethanol (2-MCE). For 5 cm cuvettes: 0.4, 1, 2, and 4 μg of AA per ml solvent as above. Prepared immediately before use.

Fractions. 70 % ethanol extracts of dried feed stuffs were treated with active charcoal, H_2S, and SE-Sephadex and passed through a column of DEAE-Sephadex.[5] The column was eluted with a 0–5 M HAc gradient containing 0.1 % 2-MCE. Fractions of 10 ml were collected. AA appeared in fractions representing approximately 2.5 M HAc.

Procedure. From each fraction three 1 ml portions are taken with an automatic transfer pipette and placed in test tubes with ground-glass stoppers. One of the tubes is used as a blank.

To each tube 2,6-DCPI is added with a medicine dropper until a reddish color persists. 0.25 ml of 2,4-DNPH reagent is added, except to the blank, using an automatic, all-glass pipetting device. After shaking, all tubes are placed in a water bath at 60°C for 1 h. The tubes are then placed in an ice bath and left for 10 min. 1.25 ml of 16 M H_2SO_4 is then added slowly to all tubes, again using an automatic all-glass pipetting device. The tubes are shaken in the ice bath. 0.25 ml 2,4-DNPH reagent is now added to the blanks while still in the ice bath. All tubes are then removed from the ice bath and allowed to stand for 30 min at room temperature. The optical density is measured at 520 nm in 1 or 5 cm cells using a Beckman model B spectrophotometer equipped with a Gilson automatic transferator. The amount of AA is calculated from a standard curve.

Results and discussion. Several modifications [6–8] of the 2,4-DNPH method have been suggested in the past, in order to simplify or speed up the reaction, but have, rightly, been criticized by Roe as impairing the specificity of the method. In the present application, strict adherence to the original procedure is less important, since coupling with 2,4-DNPH occurs only after purification of the extract and separation of ascorbic acid.

The use of 2,6-DCPI to oxidize AA to DHA, proposed by Bolin and Book,[7] is more convenient than the use of active charcoal. The 2,6-DCPI reagent is easy to prepare and dispense, and obviates the need for filtration of the sample. A clarifying agent is not required at this point, since pigments *etc.*, have been removed at an earlier stage.

The moderately elevated temperature of incubation was motivated by the desirability of shortening the incubation time, in order to fit the complete analytical procedure within a single, normal working day. Admittedly, a rise in temperature reduces the specificity of the reaction.[3,4] However, this is not a serious factor in the present procedure in view of the purity of the samples. In case of doubt on this point, the absorption spectrum of the osazone formed may readily be obtained and compared with the osazone given by synthetic AA and 2,4-DNPH. Alternatively, the identity of the osazone may be checked by thin layer chromatography.[5]

Figs. 1 and 2 show typical standard curves obtained with 1 and 5 cm cells, respectively. These curves show an essentially linear relation between optical density and the amount of AA. Using 5 cm cells, amounts down to about 0.2 μg of AA can be assayed. This makes it possible to quantitatively determine trace amounts of

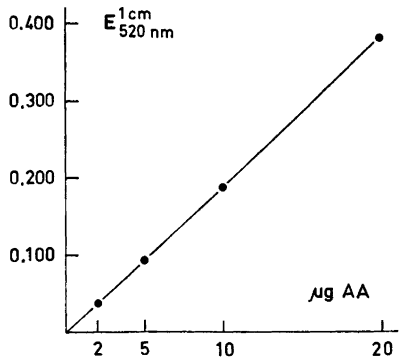

Fig. 1. Typical standard curve for amounts of ascorbic acid between 2 and 20 μg, obtained with a 1 cm cell.

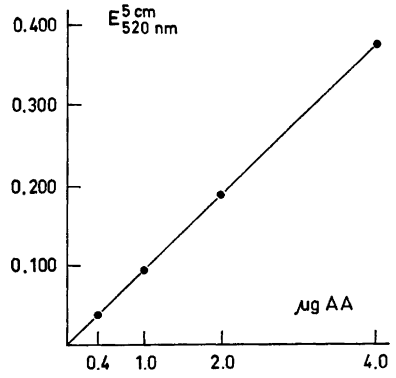

Fig. 2. Typical standard curve for amounts of ascorbic acid between 0.4 and 4 µg, obtained with a 5 cm cell.

vitamin C in dried feed stuffs down to approximately 0.1 µg/g.[5]

The modifications described in the present paper make it possible for one person to determine readily the ascorbic acid content of some 50—60 samples (150—180 tubes) per day.

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Equiatomic Transition Metal Alloys of Manganese

VII. A Neutron Diffraction Study of Magnetic Ordering in the IrMn Phase

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The purpose of the protracted research program of which the work described in this paper forms a part is to elucidate the structural and magnetic properties of transition metal phases with the CsCl and CuAu(I) types of crystal structures, especially in relation to the sizes and valences of the component atoms. The investigations [1-6] have hitherto been limited to alloys of manganese in combination with another transition element (Rh, Ir, Ni, Pd, Pt, or Au), since essentially ordered arrangements of the CsCl and CuAu(I) types are found to be stable over considerable ranges of composition and temperature in the equiatomic regions of these alloy systems. An advantage of studying these phases is that the second component has generally a very small or zero moment, which simplifies the interpretation of the magnetic data and has allowed a tentative bonding scheme to be suggested [4-6] for the phases NiMn, PdMn, and PtMn. The RhMn and IrMn phases provide further possibilities for testing and adjusting the proposed model, but most of the previous data [2,7-11] are unsuitable for this purpose. In order to obtain a partial remedy to this situation the neutron diffraction study of the IrMn phase reported here was undertaken, and the results of a detailed study of the cubic and tetragonal RhMn phases will be presented in a forthcoming paper.

Three alloys with intended compositions 45, 49, and 53 atomic % Ir were prepared from 99.9 % pure Ir (Johnson, Matthey & Co., Ltd.) and electrolytic 99.9+% pure Mn (Johnson,