Oxidation of α-Tocopherol to α-Tocopheryl Quinone by Carbon Tetrachloride-Ethanol Solvent

ABSTRACT

The addition of small amounts of CCl_4 to ethanolic solutions of α-tocopherol, vitamin A acetate or β-carotene caused destruction of these fat-soluble compounds. The oxidation product of α-tocopherol was identified as α-tocopheryl quinone by UV and IR spectral analysis. Maximum conversion to the quinone occurred with a ratio of CCl_4 to ethanol of 25:75 (v/v).

Butler (1) has shown that CCl_4 induced the non-enzymatic oxidation of reduced glutathione, cysteine and cytochrome C at physiological temperatures. Hove (2) reported that CCl_4 catalyzed the bleaching of β-carotene in ethanol solution in the presence of linoleate hydroperoxide. Recknagel and Ghoshal (3) found that a conjugated diene was formed from the linoleic acid of liver microsomes after CCl_4 treatment. Butler (1) has shown that CCl_4-induced oxidations resulted in an inverse substitution of one of the chlorine atoms, presumably through free radical formation, and assumed that the final fate of this chlorine atom was as a chloride ion produced by acquisition of two electrons from the substrate compound. He suggested that this oxidative activity may be involved in the hepatotoxic effect of CCl_4 in animals. If this is true, then antioxidants should confer protection against the toxic action of this solvent. Indeed, several years ago Hove (4) did show that α-tocopherol and other antioxidants had a marked protective effect against fatal CCl_4 poisoning of rats fed low protein diets. This finding has recently been confirmed by Gallagher (5) and by Seward et al. (6). Therefore it is of interest to find now that CCl_4 can oxidatively destroy α-tocopherol by causing its conversion to the quinone.

When glass-distilled spectral grade CCl_4 was added to ethanolic solutions of dl-α-tocopherol (70 μg/ml), conversion to the tocopheryl quinone began quickly and proceeded to near completion within a period of several days (Fig. 1). Quantitative conversion, as observed spectrophotometrically, occurred within 20 days when the CCl_4 concentration was 20% (v/v). In control solutions (no CCl_4) under the same time and conditions, no change occurred. The loss of the tocopherol was indicated by the complete disappearance of the Emmerie-Engel reaction and by loss of the UV absorption peak at 293 mμ (Fig. 2). The UV curve showed the strong bicuspid peaks at 264 and 273 mμ that are characteristic of the quinone, with $E^{1\%}_{1cm}$ (273)=345 (Fig. 3). The IR spectrum of the dry product conformed exactly to that of authentic α-tocopheryl quinone (7). In another trial (Fig. 1 and 2), $CHCl_3$ was found to be less than half as active as CCl_4 in oxidizing tocopherol to the quinone.

The rapidity and degree of oxidation of tocopherol by CCl_4 depends on the relative ratio of the two solvents, ethanol and CCl_4. In either pure CCl_4 or pure ethanol, tocopherol was quite stable. The maximum rate of oxidation occurred with 25% CCl_4 in ethanol

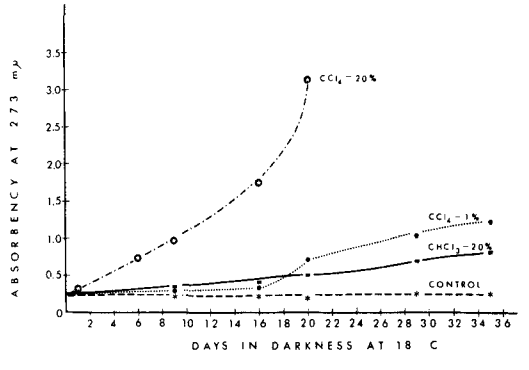

FIG. 1. UV curves showing the production of α-tocopheryl quinone (273 mμ) from α-tocopherol, induced by low levels of CCl_4 at 18 C. α-Tocopheryl quinone production was monitored by periodic UV readings over 36 days.

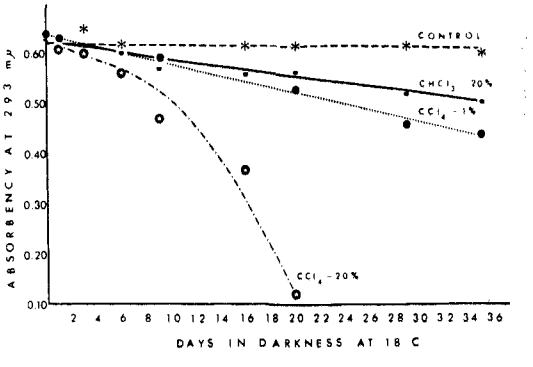

FIG. 2. UV curves showing the loss of absorption at 293 mμ (α-tocopherol) induced by low levels of CCl_4 at 18 C. α-Tocopherol loss was monitored by periodic UV readings over 36 days.

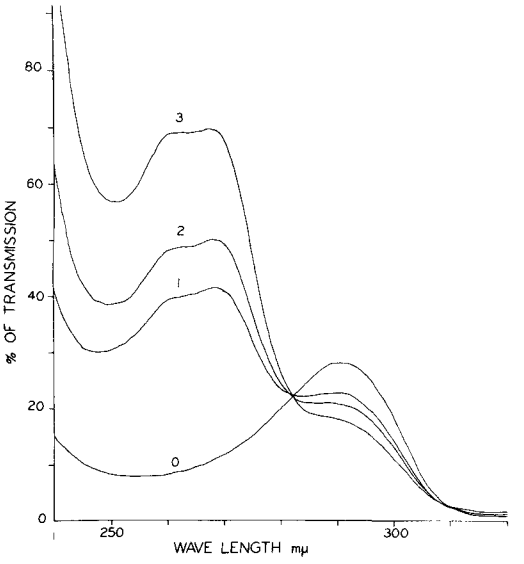

FIG. 3. UV curves showing the concomitant loss of α-tocopherol (294 mμ) and production of α-tocopheryl quinone (264, 273 mμ) induced by low levels of CCl_4 at 18 C. Volumes of CCl_4 for curves 0, 1, 2 and 3 were 0%, 1.25%, 2.50% and 5.00% in ethanol, respectively.

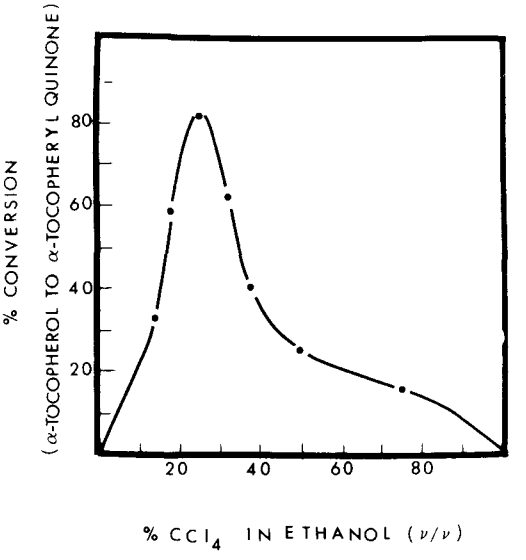

FIG. 4. Effect of proportions of CCl_4 in ethanol on the oxidation of α-tocopherol to α-tocopheryl quinone, at 18 C over a 4 day period in dim, diffuse, laboratory light.

(v/v), as shown in Figure 4.

We have obtained other data showing that vitamin A acetate is even more labile to the solvent effect than is tocopherol. As little as 1.3% of CCl_4 added to vitamin A acetate in a mixture of ethanol and xylene (11 μg/ml) caused the destruction of 53% in 4 days; control solutions with no CCl_4 were completely stable under identical conditions.

The oxidative effect of CCl_4 probably depends on free radical primers. Polar compounds such as ethanol could be such primers, and could explain the effect of the mixed solvents. We also found that diffuse laboratory light was a primer; in 4 days the destruction of β-carotene by 5% CCl_4 was 85% in light but was only 30% in total darkness. In the control solutions (without CCl_4) no loss occurred in this period, even in the light. Short wavelength UV light (254 mμ) has been found to be an especially effective primer. Under this radiation, the reaction time was minutes rather than days, but approximately the same amount of destruction occurred. We have also noted that a high level of hydroquinone (0.1 g/4.2 ml reaction mixture) almost completely inhibited the CCl_4-induced oxidations.

The oxidative potential of CCl_4 under certain conditions may have a relation to its hepatotoxic action, as has been suggested in the literature. In addition, this property has important implications for the extraction of samples and storage of samples and standards in analytical procedures for easily oxidized fats and fat-soluble components.

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REFERENCES

1. Butler, T. C., J. Pharmacol. Exptl. Therap. 134:311 (1961).
2. Hove, E. L., J. Nutr. 51:609 (1953).
3. Recknagel, R. O., and A. K. Ghoshal, Nature 210:1162 (1966).
4. Hove, E. L., Arch. Biochem. 17:467 (1948).
5. Gallagher, C. H., Aust. J. Exptl. Biol. Med. Sci. 40:241 (1962).
6. Seward, C. R., G. Vaughan and E. L. Hove, Proc. Soc. Exptl. Biol. Med. 121:850 (1966).
7. Isidorides, A., J. Am. Chem. Soc. 73:5146 (1951).

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