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THIOCTIC ACID IN WHEAT FLOUR

By W. R. MORRISON and B. R. COUSSIN

The presence of thioctic acid in wheat flour has been confirmed and a concentrated extract, which, however, contains impurities, has been prepared. The acid was identified by its ultra-violet absorption maximum at 320–328 mμ, whereas in the absence of impurities its absorption maximum is at 334 mμ. Thioctic acid was also identified as its methyl ester by gas chromatography. Comparisons were made on its distribution in the mill streams of a wheat sample.

Introduction

The rôle of disulphide- and sulphydryl-containing substances in the oxidation of wheat flour doughs has been of interest to cereal chemists for many years.[1, 2] Recently there has been evidence to suggest that the 'improving' action of oxidising agents added to doughs is through oxidation of sulphydryl groups, thereby preventing their participating in sulphydryl–disulphide exchanges which are thought to be a possible mechanism for the relaxation of strains produced by dough mixing and moulding.[3–5]

Recently Dahle & Sullivan reported[6] the tentative identification of thioctic acid (α-lipoic acid) in flour, and advanced the hypothesis that in its oxidised sulphoxide form it could act as a natural oxidant and is involved in the mechanism of flour maturing.

In the first place an attempt to repeat Dahle & Sullivan's work to identify thioctic acid in extracts from flour samples, gave inconclusive results, due primarily to oxidation during the isolation procedure, and to gross amounts of impurities. Accordingly another approach was developed for the purification of the flour extracts and the identification of thioctic acid therein, and this is reported below.

Experimental

(a) *Acid digestion of flour*

Whole wheat flour (100 g.) was digested under reflux in 500 ml. of 1N-hydrochloric acid for 3 h. After being cooled, the digest was extracted with 150 ml. of chloroform taking care to avoid emulsification. (Chloroform is preferred to benzene or light petroleum because thioctic acid and its sulphoxide are more soluble in it.[7]) The crude chloroform extract was concentrated to dryness under nitrogen for reduction and methylation.

(b) *Reduction of thioctic acid sulphoxide*

Experiments with synthetic thioctic acid sulphoxide dissolved in methanol at 50° showed that 90% of it could be reduced to thioctic acid by sodium borohydride in 2 h. Complete reduction required a much longer time, but for convenience 2 h. was used for reduction of the crude extract. Thioctic acid has an absorption maximum at 334 mμ,[8, 9] but the sulphoxide has no absorption maximum in this region—this provides an easy method for measuring the degree of oxidation or reduction of a pure sample.

After 2 h. the reduction was stopped by addition of hydrochloric acid, and the mixture partitioned between water and chloroform. The chloroform layer was dried with anhydrous sodium sulphate and evaporated to dryness under nitrogen for methylation.

(c) *Methylation*

The boron fluoride–methanol reagent described by Metcalfe & Schmitz[9] was tried with synthetic thioctic acid. Apart from some manipulative loss, the method appears to be satisfactory as measured by the ultra-violet absorption at 334 mμ. The esters were isolated in light petroleum (b.p. below 40°), dried with anhydrous sodium sulphate and evaporated to dryness under nitrogen for vacuum distillation.

(d) *Vacuum distillation*

Distillation was carried out in an 18 cm. × 2·5 cm. diameter test-tube fitted with a short 2 cm. diameter side-arm leading to the vacuum line. A cold finger condenser was fitted into the tube with a clearance of 1 cm. between the tip of the condenser and the bottom of the tube. Trial distillations were made with a mixture of methyl esters of thioctic acid and fatty acids in the range C_{12}–C_{20} and the distillates analysed by gas chromatography. Comparison with the undistilled mixture showed that methyl thioctate distils in a manner similar to that of methyl laurate (C_{12}). From the data of Kaufmann[10] it was deduced that methyl thioctate should boil, and therefore distil almost instantaneously, at 60°/0·1 mm., and this was found to be so. The conditions used for distillation of the natural extract were 50° for half an hour at 0·05 mm. Hg (McLeod gauge), with the cold finger condenser water at 11–12°, although milder conditions would probably have sufficed.

The distillate was washed off the cold finger with light petroleum, chloroform or any suitable solvent for spectrophotometric analysis. Synthetic methyl thioctate sulphoxide does not appear to distil even at 90°/0·01 mm., and it is thus necessary to reduce extracts prior to distillation of methyl esters.

(e) *Spectrophotometric analysis*

Synthetic thioctic acid has an absorption maximum at or about 334 mμ, depending on the solvent used. This peak tends to shift to about 330 mμ with dilution as can be seen in the results published by Cavina *et al.*,[8] and this has been verified experimentally. Distillates obtained from wheat show a broad peak at 320–325 mμ with considerable background absorption. Addition of synthetic thioctic acid increased the peak, but only gave a slight peak shift from 322 to 326 mμ. When a crude extract was distilled without prior reduction, no absorption maximum near 334 mμ was found, with or without reduction of the distillate. This is consistent with the behaviour of synthetic methyl thioctate sulphoxide. We believe that the peak found in the distillate of the reduced methylated extract is due to methyl thioctate, and that the shift from near 334 mμ to 320 mμ is due partially to the dilution effect, but mainly to impurities in the distillate.

(f) *Gas chromatography*

Gas chromatographic analyses were made with Pye Argon Gas Chromatography units with non-polar and polar liquid phases. The non-polar unit consisted of 10% Apiezon L stopcock grease on 60–85 mesh Embacel, column temperature 197° and argon flow rate 53·6 ml./min. The polar unit consisted of 15% LAC-2-R446 (linear polyester of ethylene glycol and adipic acid, cross-linked with pentaerithritol) with 2% phosphoric acid on 80–100 mesh Celite, column temperature 182° or 192° and argon flow rate 40 ml./min.

Synthetic methyl thioctate was found to have a carbon number of about 15·5 in the non-polar column, and 21·2–21·4 in the polar column. Chromatography of the distillates obtained from whole wheat flour showed the presence of considerable amounts of fatty acid esters, probably from the free fatty acids of the flour, and small amounts of their oxidation products. It was thus not possible to identify methyl thioctate with any certainty by gas chromatography in a non-polar column because of the impurities in the distillate. However, in the polar column a small peak corresponding to methyl thioctate was found, and it was well separated from all other esters of carbon number up to ~19·7. It is possible that this peak could be due to methyl arachidonate, but the analysis on the non-polar column showed no ester of an acid of carbon number 19 or higher. Distillates which had not been reduced prior to distillation, and which showed no absorption maximum at 320–325 mμ, gave no peak corresponding to methyl thioctate on gas chromatographic analysis.

Results

No quantitative results have yet been achieved, but some comparisons of a roughly quantitative nature have been made. High-grade spring wheat flour appears to contain little thioctic acid, whereas bran and germ (defatted with light petroleum) contain measurable amounts. In an experiment with 50 g. of dried yeast the recovery of methyl thioctate was greater than from 200 g. of whole wheat flour. It is also interesting to note that in the case of the yeast extract the contaminating material in the extracts was always much less, and the absorption maximum had only shifted to 328 mμ. Gas chromatography also showed much less impurities of carbon number 16–19.

Conclusions

A preliminary separation and identification of thioctic acid as its methyl ester from wheat products and dried yeast has been achieved. The behaviour of methyl thioctate on vacuum distillation and in gas chromatography has been described for the purpose of its further identification. Future work will be directed towards obtaining purer extracts from wheat flour, and putting the procedure on to a quantitative basis.

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[*Authors' note.*—Since this paper was prepared for publication Sullivan, B., Dahle, L. K., & Peterson, D. A. (*Cereal Chem.*, 1961, **38,** 463–465) have isolated thioctic acid from wheat flour, and report 1–10 p.p.m. in flour and 200–300 p.p.m. in wheat germ. Our comparison of the relative amounts of thioctic acid in the mill streams of a wheat sample would indicate a distribution ratio of that order.]

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INSECTICIDAL ACTIVITY OF PYRETHRUM EXTRACT AND ITS FOUR INSECTICIDAL CONSTITUENTS AGAINST HOUSE FLIES. II.*—Synergistic Activity of Piperonyl Butoxide with the Four Constituents

By R. M. SAWICKI

The toxicity to female house flies, 5–6 days old, of the four insecticidal constituents of pyrethrum, combined with piperonyl butoxide at six ratios (from 10 : 1 to 1 : 20 w/w), was determined by a measured drop technique. The order and magnitude of the toxicities of the synergised constituents (relative to synergised pyrethrum extract) vary with the amount of synergist present. At 1 : 8 (pyrethroid/piperonyl butoxide) the relative toxicities of the four constituents were: pyrethrum extract, 1·0 (1·0); pyrethrin I, 1·31 (0·85); cinerin I, 1·28 (0·53); pyrethrin II, 0·90 (1·37); cinerin II, 0·58 (0·49). (The relative toxicities of the constituents alone are given in brackets.) At this ratio, the synergistic factors of the four constituents were: pyrethrin I, 18; cinerin I, 27; pyrethrin II, 6; cinerin II, 14; pyrethrum extract, 10. The possible explanations for differences of the extent to which the constituents are synergised are discussed.

Introduction

The only paper on the synergistic effects of piperonyl butoxide with the four insecticidal constituents of pyrethrum taken separately is that of Incho & Greenberg.[1] Their results, obtained by the Campbell & Sullivan turn-table method,[2] are difficult to interpret, because the amount of poison picked up by the flies is not known and may have varied with the substances tested. The quantity of insecticide picked up depends, among other things, on the activity of the insects during treatment and on the knockdown speed of the compounds tested. A measured-drop technique determines the activity per unit weight of the four synergised constituents of pyrethrum accurately and avoids the uncertainties mentioned above. This technique was therefore used to determine the absolute and the relative increases in the toxicities of the four constituents in the presence of piperonyl butoxide and the changes in the order and magnitude of the relative toxicities of the four constituents at various ratios of pyrethroid to synergist.

Materials and methods

The toxicity tests followed the general design of probit assays.[3] The full details of the bio-assay technique have been described elsewhere.[4] Female house flies, usually 5–6 days old at the time of treatment, were each dosed, by a measured drop technique, with one 1-μl. drop

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* Part I: *J. Sci. Fd Agric.*, 1962, **13,** 172