CYCLOPROPENYL COMPOUNDS AS SULFHYDRYL REAGENTS

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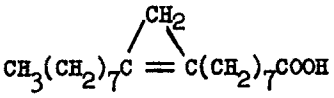
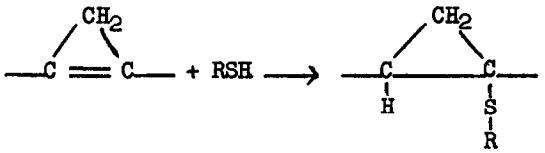
Cyclopropenyl fatty acids (CPA) occur in a small number of species of plants. The best known of these fatty acids, sterculic acid, ω-(2-n-octyl-cyclopropen-1-yl)octanoic acid, is the major component of Sterculia foetida oil:



The lipids of Gossypium hirsutum contain small quantities of cyclopropenyl fatty acids, principally ω-(2-n-octyl-cyclopropen-1-yl)heptanoic acid, commonly called malvalic acid. These acids upon ingestion produce a number of biological effects which include altered membrane permeability of eggs, elevated stearic acid levels of yolk, heart, plasma, liver, and ovary fat of hens, and body fats of swine at the expense of oleic acid (Evans et al., 1962; Shenstone and Vickery, 1959; Masson et al., 1957; Ellis et al., 1931). Although the mode of action of these compounds is not known, one possibility is that the cyclopropene ring will react with mercaptans to bind the sulfur in a thioether linkage:



Such a reaction could irreversibly inactivate enzymes requiring free SH groups. Kircher (1964) demonstrated the reactions of mercaptans with

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methyl sterculate and sterculene (1,2-dioctylcyclopropene), and suggested that the same type of reaction might take place *in vivo*.

The particulate acid lipase of *Ricinus communis* is reversibly inhibited by mercurials (Ory *et al.*, 1960). It was of interest, therefore, to test the hypothesis that the cyclopropene ring could inhibit this SH enzyme.

The results with *Sterculia foetida* oil as the source of cyclopropenes are given in Table 1. Lipolysis was reduced by 50 percent under the conditions reported; there was no inhibition if both the incubation with *Sterculia foetida* oil and lipolysis took place in the presence of cysteine. If cysteine was added after a 20 minute period of incubation with inhibitor, there was only partial countering of inhibition. Cysteine apparently reacts preferentially with cyclopropenes. When cysteine is added after incubation of enzyme and inhibitor, it can only react with the unreacted cyclopropenes, hence the inhibition is only partially countered.

Table 1. Inhibition by *Sterculia foetida* oil of the acid lipase of *Ricinus communis*.

S. foetida oil (mg.)	Cysteine (μmole)	CPA (initial) (μmole)	Fatty acids released (μ mole/10 min.)
-	-	-	35.8
-	4.5	-	37.8
2	-	3.3	16.9
2	4.5	3.3	34.6
2	4.5*	3.3	23.7

1.3 mg. enzyme protein (Preparation A), 1000 μmole substrate, inhibitor, cysteine, and water to 4.8-4.9 ml. were homogenized for 1 min., poured into a reaction vessel and stirred under nitrogen at 25°C., and pH 6.6-7.0 for 20 min. Acetic acid was then added to pH 4.2; extent of lipolysis was determined by measuring the increase in titratable acidity in 5 ml. of reaction mixture (Altschul *et al.*, 1963). The sterculic acid content (reported as CPA) of the *S. foetida* oil was determined according to Harris *et al.*, (1964).

*After all ingredients except cysteine were mixed together for 20 min., cysteine was added and the incubation continued for 10 min. more followed by enzyme assay.

A sample of lipids of Gossypium hirsutum selected for its high content of CPA was hydrolyzed slower than one containing lower concentrations of this acid (Table 2). The rate of lipolysis of these oils was increased if cysteine was added.

Table 2. Effect of malvalic acid content on hydrolysis of lipids of Gossypium hirsutum by castor lipase.

Substrate	Cysteine (μmole)	CPA (Initial) (μmole)	Fatty acids released (μmole/10 min.)
Sample 1	-	2.7	19.2
	4.5	2.7	26.3
Sample 2	-	10.1	12.4
	4.5	10.1	18.9

Conditions same as for Table 1; the enzyme was Preparation A. Sample 1 was a commercial refined and bleached cottonseed oil. Sample 2 was the lipids extracted with petroleum ether from seeds of particularly high malvalic acid content and purified to remove phospholipids, non-esterified fatty acids, and pigments by passage over a column of activated alumina (AOCS official method Ca 9f-57). Malvalic acid content was determined according to Harris et al., (1964).

Confirmation that the inhibition observed with mixtures of natural lipids could be attributed to the cyclopropene moiety was obtained by results with sterculene shown in Table 3. Contact between inhibitor and enzyme either in the presence of substrate, as in the other experiments, or for a short period in the absence of substrate, gave comparable results.

The inhibition of Ricinus lipase by CPA and sterculene suggests that similar inhibition might be observed with other SH enzymes. Lynen (1961) and Wakil (1962) emphasized the role of SH enzymes in the biosynthesis of fatty acids. Therefore, the presence of a cyclopropene moiety in a lipid component of a diet could conceivably interfere with lipid metabolism.

These results also suggest a possible analytical procedure for biologically active cyclopropenyl compounds by measuring the inhibition of

Table 3. Effect of sterculene on hydrolysis of triolein by castor lipase.

Lipase preparation	Sterculene (μmole)	Cysteine (μmole)	Fatty acids released (μmole/10 min.)
A	-	-	25.8
A	10.3	-	8.2
A	18.7	-	6.1
B	-	-	22.0
B	3.8	-	11.0
B	3.8	4	20.2
C	-	-	36.0
C	3.8	-	0
C	3.8	4	28.4

Conditions for test with lipase Preparation A same as for Table 1. Lipase Preparations B or C, inhibitor, cysteine, and water were homogenized 1 min., then substrate was added and homogenization continued for 1 min. Acetic acid was then added to pH 4.2 to initiate lipolysis.

Ricinus lipase activity. Moreover, since the bond formed between the cyclopropene ring and protein thiol groups would resist acid hydrolysis, this offers the possibility of another means for labeling the functional cysteine residues of SH-sensitive enzymes. Since glycerides containing sterculic and malvalic acids are quite similar to the natural substrates of this lipase (e.g., triolein), there is the added possibility of competitive inhibition, aside from the specific reaction with the SH site(s). These aspects are being investigated on a solubilized and purified *Ricinus* lipase preparation.

Acknowledgment

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