where $T_1$ and $T_2$ are, respectively, the temperatures for 10% and 90% conversion and $\Delta H_{\text{m}}$ is the molar enthalpy of the transition in kcal per mole of cooperative units. In our instrument a transition having a width in excess of 50° would be detectable at a concentration level of several milligrams per ml.

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**REFERENCES**

1. Engelman, D. M. (1970) J. Mol. Biol. 47, 115-117
2. Stein, J. M., Tottelotte, M. E., Reinert, J. C., McEwaney, R. N., and Raden, R. L. (1969) Proc. Nat. Acad. Sci. U. S. A. 63, 104
3. Melchior, D. L., Morowitz, H. J., Steurtevant, J. M., and Tong, T. Y. (1970) Biophys. Biophys. Acta 190, 114-122
4. Ladbroke, B. D., Williams, R. M., and Chapman, D. (1968) Biophys. Biophys. Acta 150, 333-340
5. Danforth, R., Kharait, H., and Steurtevant, J. M. (1967) Res. Sci. Inst. 35, 494
6. Tong, T. Y., Hearn, R. P., Warthall, D. F., and Steurtevant, J. M. (1970) Biochemistry 9, 2068
7. Methods for Chemical Analysis of Water and Wastes, Federal Water Pollution Control Administration, November 1969
8. Ladbroke, B. D., and Chapman, D. (1969) Chem. Phys. Lipids 3, 304
9. Chapman, D., Williams, R. M., and Ladbroke, B. B. (1967) Chem. Phys. Lipids 4, 445
10. Engelman, D., and Rothman, J. (1972) J. Biol. Chem. 247, 3094-3097
11. Leduc, H., and Derbey, D. G. (1971) J. Mol. Biol. 55, 59-57
12. Oldfield, E., and Chapman, D. (1971) Biochem. Biophys. Res. Commun. 360, 610-616
13. Oldfield, E., Chapman, D., and Derbyshire, W. (1971) Fed. Eur. Biochem. Soc. Lett. 15, 102
14. Darke, A., Finer, E., Flocco, A. G., and Phillips, M. C. (1972) J. Mol. Biol. 62, 285
15. Badley, R., A., Schneider, H., and Martin, W. G. (1971) Biochem. Biophys. Res. Commun. 45, 174
16. Heia, J.-C., Schneider, H., and Smith, I. C. P. (1971) Biochem. Biophys. Res. Commun. 49, 614-622
17. Lippert, J. L., and Peticolas, W. L. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1572

**Summary**

A recent report (J. Biol. Chem. (1970) 245, 1856) that the inhibition of the aerobic desaturation of stearoyl coenzyme A by sterculate is a nonspecific detergent effect was reinvestigated. It was found that whereas 0.04 mM potassium sterculate inhibited about 50% of mouse liver microsomal desaturation activity, it required 0.3 mM concentration of potassium oleate to effect the same level of inhibition. Also, the degree of inhibition of the desaturase by sterculate was found not to be dependent on the protein concentration. Thus the specific effect of cyclopropene fatty acids in the inhibition of the stearoyl-CoA desaturase is confirmed.

**Inhibition of Stearoyl Coenzyme A Desaturase by Sterculate in Mouse Liver Microsomes**

(Published for publication, March 24, 1972)

**REFERENCES**

1. Engelman, D. M. (1970) J. Mol. Biol. 47, 115-117
2. Stein, J. M., Tottelotte, M. E., Reinert, J. C., Mcewaney, R. N., and Raden, R. L. (1969) Proc. Nat. Acad. Sci. U. S. A. 63, 104
3. Melchior, D. L., Morowitz, H. J., Steurtevant, J. M., and Tong, T. Y. (1970) Biophys. Biophys. Acta 190, 114-122
4. Ladbroke, B. D., Williams, R. M., and Chapman, D. (1968) Biophys. Biophys. Acta 150, 333-340
5. Danforth, R., Kharait, H., and Steurtevant, J. M. (1967) Res. Sci. Inst. 35, 494
6. Tong, T. Y., Hearn, R. P., Warthall, D. F., and Steurtevant, J. M. (1970) Biochemistry 9, 2068
7. Methods for Chemical Analysis of Water and Wastes, Federal Water Pollution Control Administration, November 1969
8. Ladbroke, B. D., and Chapman, D. (1969) Chem. Phys. Lipids 3, 304
9. Chapman, D., Williams, R. M., and Ladbroke, B. B. (1967) Chem. Phys. Lipids 4, 445
10. Engelman, D., and Rothman, J. (1972) J. Biol. Chem. 247, 3094-3097
11. Leduc, H., and Derbey, D. G. (1971) J. Mol. Biol. 55, 59-57
12. Oldfield, E., and Chapman, D. (1971) Biochem. Biophys. Res. Commun. 360, 610-616
13. Oldfield, E., Chapman, D., and Derbyshire, W. (1971) Fed. Eur. Biochem. Soc. Lett. 15, 102
14. Darke, A., Finer, E., Flocco, A. G., and Phillips, M. C. (1972) J. Mol. Biol. 62, 285
15. Badley, R. A., Schneider, H., and Martin, W. G. (1971) Biochem. Biophys. Res. Commun. 45, 174
16. Heia, J.-C., Schneider, H., and Smith, I. C. P. (1971) Biochem. Biophys. Res. Commun. 49, 614-622
17. Lippert, J. L., and Peticolas, W. L. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1572

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For the Department of Biochemistry and Biophysics, Texas Agricultural Experiment Station, College Station, Texas 77843

**Summary**

A recent report (J. Biol. Chem. (1970) 245, 1856) that the inhibition of the aerobic desaturation of stearoyl coenzyme A by sterculate is a nonspecific detergent effect was reinvestigated. It was found that whereas 0.04 mM potassium sterculate inhibited about 50% of mouse liver microsomal desaturase activity, it required 0.3 mM concentration of potassium oleate to effect the same level of inhibition. Also, the degree of inhibition of the desaturase by sterculate was found not to be dependent on the protein concentration. Thus the specific effect of cyclopropene fatty acids in the inhibition of the stearoyl-CoA desaturase is confirmed.

Inhibition of stearic to oleic acid conversion by cyclopropene fatty acids was demonstrated from our laboratory (1, 2) in rats with both in vitro and in vivo experiments. This phenomenon was confirmed by several others (3-6). Recently Pande and Mead (7) reinvestigated this effect and reported that the inhibition of the stearoyl coenzyme A desaturase by sterculate is nonspecific and probably due to the detergent action of the fatty acids. However, these workers did find that microsomes isolated from rats fed methyl sterculate showed low desaturase activity.

The concentration of sterculate required to obtain about 50% inhibition of the desaturase activity in Pande and Mead's experiments is about 10 to 100 times higher than the concentrations used by James et al. (6) and Johnson et al. (5). Even though the assay systems vary widely, the very large difference in the amount of sterculate required for appreciable inhibition of the desaturase casts doubt upon the purity of the sterculate used by Pande and Mead. This contention is strengthened by the fact that the sources of sterculic acids were different in in vivo and in vitro experiments. It should be noted that sterculyl alcohol and 2-hydroxy sterculyl alcohol have also been found to be potent inhibitors of the stearoyl desaturase activity (5, 6). The present study was undertaken to re-examine the phenomenon by comparison of the effects of authentic sterculate and oleate on stearoyl-CoA desaturase in mouse liver microsomes.

Methyl sterculate was isolated from Sterculia foetida seed oil by the urea adduct method procedure of Kircher (8). Gas-liquid chromatographic analysis of the silver nitrate-methanol reaction products (9) and ethyl mercaptan addition products (10) showed that the product was about 98% cyclopropene fatty acid esters, of which about 2% was methyl malvalate. The non-cyclopropene fatty acid impurities were mainly oleate and linoleate. Nuclear magnetic resonance spectra of the methyl sterculate preparation in CCl4 showed the characteristic peak at 9.27 ppm. The relative areas of propene and methoxy hydrogen peaks were found to be 2:3, suggesting a high degree of purity of the cyclopropene fatty acid ester.

The potassium salt of the sterculic acid was prepared by mixing the methyl sterculate with 2 eq of potassium hydroxide in alcohol for 2 hours in a Vortex mixer under a nitrogen atmosphere. The alcohol was evaporated by a stream of nitrogen and the soap was diluted with distilled water to the desired concentation of potassium sterculate.

**Table I**

| Concentration (mM) | Oleate produced (nmol) | Inhibition (%) |
|-------------------|------------------------|---------------|
| 0                 | 4.2                    | 11            |
| 0.04              | 3.56                   | 34            |
| 0.2               | 2.90                   | 46            |

*This work was supported by grants from the National Institutes of Health (AM 0601). The Texas Agricultural Experiment Station, and The National Dairy Council.
tration. Fresh preparations of potassium sterculate were used in each experiment. Potassium oleate was prepared similarly from methyl oleate (99% pure) obtained from the Hormel Institute, Austin, Minnesota.

Table I shows the effect of potassium sterculate and potassium oleate on the stearoyl-CoA desaturase in mouse liver microsomes. Forty-six per cent inhibition of the desaturase was effected by potassium sterculate at a concentration of 0.04 mM, whereas it required 0.3 mM concentration of oleate to produce the same level of inhibition. Also 0.1 mM sterculate produced an inhibition of about 77%, whereas the same level of oleate produced only 11% inhibition.

The concentration of sterculate required to give about 50% inhibition of the desaturase system in the present study was only about one-tenth of that used by Pande and Mead (7). Also the extent of inhibition by oleate was only about one-seventh of that of sterculate, showing an effect by sterculate not shared by oleate. The inhibition of the desaturase at 0.3 mM concentration of sterculate may be partly due to a nonspecific detergent effect.

If the inhibitory effect of sterculate on the desaturase is a nonspecific detergent action, then the inhibition in vitro should decrease with increase in enzyme concentration (Table II). However, the levels of inhibition were about the same, whether 77 or 231 μg of microsomal proteins were used. This shows that the detergent type of action of sterculate is not effective at the 0.04 mM level.

It is relevant to note that Nordby et al. (11) have reported that sterulene (1,2-dioctyl cyclopropane) when fed to laying hens produced the same physiological effects in eggs as methyl sterulyl ether, sterulyl alcohol, and Sterculia foetida seed oil fatty acid methyl esters. Thus a fatty acid moiety is not essential for the physiological effect of cyclopropene fatty acids.

That stearoyl desaturase is a sulfhydryl-sensitive enzyme has been shown (2, 12, 13). Reaction of sterulic acid with thiol compounds is well documented by the works of Kircher (8), Raju and Reiser (10), and Hooper and Law (14). The inhibition of castor bean lipase by sterulic acid has been shown to be due to the —SH binding (15). These studies suggest that the inhibition of the stearoyl-CoA desaturase system by sterculate may be due to the —SH binding. However, conclusive evidence for the —SH binding mechanism must await purification of the desaturase system.

### Table II

| Microsomal protein | Stericate* | Oleate formed (moles) | Percentage of inhibition |
|--------------------|-----------|----------------------|-------------------------|
| 77 μg              | -         | 1.88                 | 41                      |
| 77 μg              | +         | 1.12                 | 41                      |
| 231 μg             | -         | 7.4                  | 45                      |
| 231 μg             | +         | 4.1                  | 45                      |

* Potassium sterculate was added at 0.04 mM concentration.

REFERENCES

1. Reiser, R., and Raju, P. K. (1964) Biochem. Biophys. Res. Commun. 17, 8
2. Raju, P. K., and Reiser, R. (1967) J. Biol. Chem. 242, 379
3. Allen, E., Johnson, A. R., Fogerty, A. C., Pearson, J. A., and Shenstone, F. S. (1967) Lipids. 2, 419
4. Donaldson, W. E. (1967) Biochem. Biophys. Res. Commun. 27, 681
5. Johnson, A. R., Fogerty, A. C., Pearson, J. A., Shenstone, F. S., and Reihsten, A. M. (1969) Lipids 4, 255
6. James, A. T., Harries, A., and Baxand, J. (1968) Eur. J. Biochem. 5, 318
7. Pande, S. V., and Mead, J. F. (1970) J. Biol. Chem. 245, 1856
8. Kircher, H. W. (1964) J. Amer. Oil Chem. Soc. 41, 4
9. Schneider, E. L., Locke, S. P., and Hopkins, D. T. (1968) J. Amer. Oil Chem. Soc. 45, 585
10. Raju, P. K., and Reiser, R. (1966) Lipids 1, 10
11. Nordby, E., Heywood, R. W., Kircher, H. W., and Kemmerer, A. R. (1962) J. Amer. Oil Chem. Soc. 39, 183
12. Jones, P. D., Holloway, P. W., Puleffo, R. O., and Wakil, S. J. (1969) J. Biol Chem. 244, 744
13. Holloway, P. W., and Wakil, S. J. (1970) J. Biol. Chem. 245, 1862
14. Hooper, N. K., and Law, N. H. (1968) J. Lipid Res. 9, 270
15. Ory, R. L., and Altschul, A. M. (1964) Biochem. Biophys. Res. Commun. 17, 12
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*J. Biol. Chem.* 1972, 247:3700-3701.

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