Euphorbia lagascae Spreng. Enzyme Activity in the Seed

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Abstract

The enzyme system of *Euphorbia lagascae* Spreng. seed was found to be somewhat similar to that of *Vernonia anthelmintica* (L.) Willd. seed. The enzymes which were active only after the seed was crushed were deactivated by steam heat without alteration of the epoxy content of the oil. Enzyme activity was accelerated to produce changes in the composition of the oil as evidenced by changes in free fatty acid content, iodine values, and oxirane oxygen (epoxy) content, but the changes were not as great in *Euphorbia* as in *Vernonia*. Thin-layer chromatographic studies showed that dihydroxyoleic acid was formed during incubation of crushed seed indicating the presence of a hydrating principle in *Euphorbia*.

Introduction

EUPHORBIA LAGASCAE seed contains at least two active principles of an enzymatic nature similar to those found in *Vernonia anthelmintica* (L.) Willd. seed (1,2,4). These are the hydrolytic and the hydrating principles. An oxidative and a dehydrating principle (1,2) may be present also but the authors do not plan to investigate their presence because developmental studies on new crops have been terminated at this Laboratory.

This paper discusses the behavior of the hydrolytic and the hydrating principles of *E. lagascae* and compares their activity to that found in *V. anthelmintica* seed. As in *Vernonia*, these principles do not seem to be active in the whole seed but only after the seed covering is broken by some form of crushing.

Experimental

Analytical Methods

The source of seed, analytical methods, extraction and thin-layer chromatographic techniques used in these studies were described previously (3–5).

Deactivation of Enzymes

Steam heat was used because it had been found superior to dry heat in our work on *Vernonia* (1). The seed was heated in a steam autoclave at 15 lb pressure for 15–20 min.

Activation of Enzymes

Since a small rolling mill was not available and the high oil content of the seed made the use of an ordinary grinding mill impractical, a technique for preparing crushed seed for the activation studies was devised. Individual 40 g samples of seed were macerated in a Waring blendor, transferred to a sheet of aluminum foil on a flat, hard surface, rolled by hand until all the seeds were completely crushed, and then transferred to the appropriate container. Samples in one experiment were sealed under nitrogen in individual flasks so that the available moisture was limited to the 4.5% naturally present in the seed. Samples in the second experiment were placed in open wide-mouthed bottles and covered loosely with aluminum foil; the bottles were placed over water in a vacuum desiccator and the air was replaced by nitrogen. This provided the seed with a water-saturated nitrogen atmosphere. In both experiments the samples were incubated at 28C. Each week for 9 wk a sample from each experiment, water-saturated atmosphere and limited moisture, was extracted with a 50-50 mixture of petroleum ether-ethyl ether. Each extract was concentrated on a rotating evaporator and analyzed for free fatty acid and oxirane oxygen content and iodine value. In addition, the moisture content of crushed seed incubated in a water-saturated atmosphere was determined at the end of the ninth week.

Thin-Layer Chromatographic Studies

Silica Gel G was used as the absorbent and, unless otherwise stated, the plates were developed in Skellysolve F, ethyl ether and acetic acid, 60:40:2, v/v/v.

A comparison by thin-layer chromatography (TLC) was made of the oils obtained from the experiment in which the moisture content of the seed was constant during the 9-wk incubation period and then one of the samples, the fourth-week oil, was chromatographed on a plate with the following samples: *Euphorbia* oil obtained from freshly crushed seed; oil obtained from seed incubated for 9 weeks in a water-saturated atmosphere; a standard solution containing tripalmitin, oleic acid, 1,3-dipalmitin and monopalmitin; and a standard solution containing trivernolin, vernolic acid, 1,3-divernolin and (+) *threo*-12,13-dihydroxyoleic acid.

Eight samples, the second through the ninth-week oils, from the experiment in which the atmosphere was water-saturated were chromatographed on the same plate with (+) *threo*-12,13-dihydroxyoleic acid and developed with a more polar solvent mixture, 25:75:2, Skellysolve F, ethyl ether, acetic acid, v/v/v, to illustrate the development of the dihydroxy acid with time of incubation.

Results and Discussion

The increase in free fatty acid (FFA) content of the oils obtained from seed that was aged after crushing, as shown in Table I, demonstrates the presence of a hydrolytic principle. The increase is not as great as that with *V. anthelmintica* seed oils (1), and may be due to a less active enzyme system in *Euphorbia*. However, in *Vernonia* it was found that there appeared to be a relation between the moisture content of the seed and the rate of hydrolysis. The moisture content of the *Euphorbia* seed from which these data were obtained was 4.5%; this may account for the apparent lesser activity.

TABLE I
Effect of Aging on Crushed Seed

Crushed seed aged	FFA (as epoxyoleic)
days	%
0 [a]	0.9
4 [b]	4.9
7 [b]	17.0
7 [c]	2.1

[a] Extracted and crushed simultaneously in a Waring blendor.
[b] Exposed to room conditions for stated period before it was extracted.
[c] Autoclaved prior to being crushed and exposed to room conditions for stated period before it was extracted.

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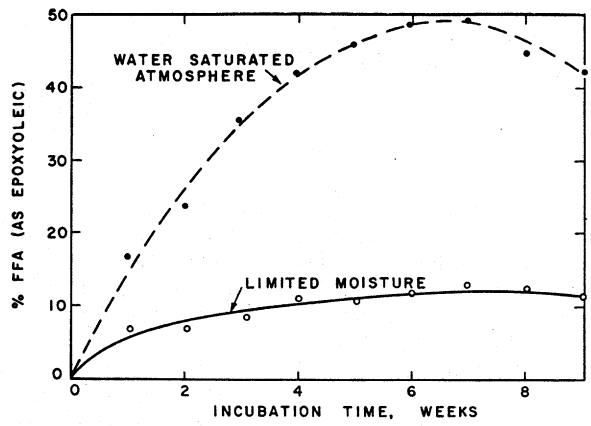

FIG. 1. The effect of time on the FFA content of oils prepared from seed incubated with limited moisture and in a water-saturated atmosphere.

Since our work on *Vernonia* (1) had already shown that steam heat was superior to dry heat as an agent to deactivate the enzyme system, it was not deemed necessary to repeat these experiments with *Euphorbia*. In order to show that the enzyme activity in *Euphorbia* could be deactivated, some seed was autoclaved prior to crushing and aging. As shown in Table I, the steam heat effectively reduced the enzyme activity. As with *Vernonia*, autoclaving did not have any effect on the epoxy content of the *Euphorbia* seed oil.

The effect of time on the properties of oils prepared from seed incubated with a limited amount of moisture and in a water-saturated atmosphere is illustrated in Figures 1, 2 and 3. The changes in FFA and oxirane oxygen content and in iodine values follow a trend similar to that exhibited by *V. anthelmintica* seed (1), but the magnitude of the changes is not as great. In fact, the changes are relatively small in the samples incubated with the moisture content limited to that naturally present in the seed. At the end of the ninth week, the moisture content of the *V. anthelmintica* seed incubated in a water-saturated atmosphere was 16.2% while that of *E. lagascae* seed treated in the same manner was 12.1%.

By TLC, the oils obtained from seed incubated with limited moisture varied little in composition. A chromatogram of the fourth-week oil is shown in Figure 4 with chromatograms of the original *Euphorbia* oil, the ninth-week oil from seed incubated in a water-saturated atmosphere, and two standards. The original *Euphorbia* oil contained nonepoxidized triglyceride, trivernolin, and three other substances, two of which migrated to positions between that of the nonexpoxidized triglyceride and that of trivernolin, and

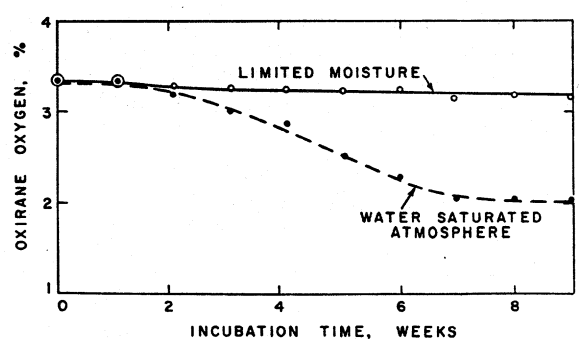

FIG. 2. The effect of time on the oxirane oxygen content of oils prepared from seed incubated with limited moisture and in a water-saturated atmosphere.

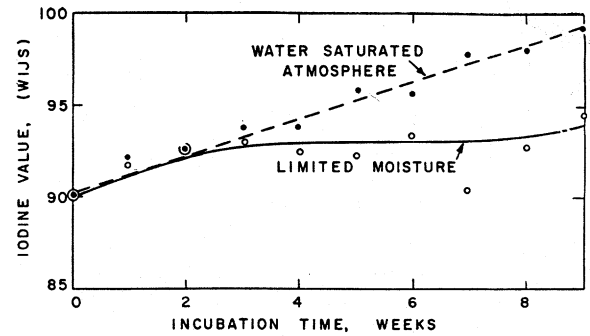

FIG. 3. The effect of time on the iodine values of oils prepared from seed incubated with limited moisture and in a water-saturated atmosphere.

one which migrated to a position between that of nonepoxidized diglyceride and that of divernolin. Their positions on the chromatogram and the fact that the vernolate has a random or restricted random distribution in *Euphorbia* oil glycerides (6) make it reasonable to speculate that the two compounds between the nonepoxidized triglyceride and trivernolin were partially epoxidized triglycerides, one containing one vernolate radical and the other two vernolate radicals and that the one between the nonepoxidized diglyceride and divernolin was partially epoxidized diglyceride. This speculation is somewhat confirmed by Kleiman's work (6) in which he separated the triglycerides of *Euphorbia lagascae* oil into four distinct bands and showed the composition of each band to be as follows: nonepoxidized triglycerides, 31% vernolate in triglyceride, 63% vernolate in triglyceride, and trivernolin (98% vernolate).

The fourth-week oil from the experiment in which the moisture content of the seed was constant contained nonepoxidized triglyceride, the two compounds speculated to be partially epoxidized triglycerides with one and two vernolate radicals respectively, trivernolin, the compound speculated to be partially epoxidized diglyceride, divernolin, and some material that appears to be a mixture of vernolic acid and nonepoxidized diglyceride. Although it does not appear in the chromatogram in Figure 4, other chromatograms of this fourth-week oil indicated that it also contained a trace of nonepoxidized fatty acid.

The chromatogram of the oil obtained from seed incubated for 9 wk in a water-saturated atmosphere

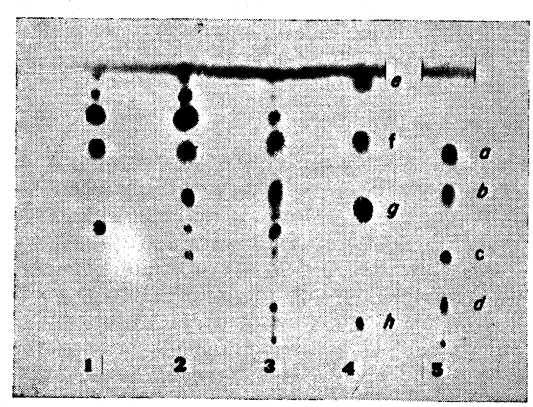

FIG. 4. TLC of *Euphorbia* oil samples. 1) Original *Euphorbia* oil; 2) Fourth-week oil from seed incubated with limited moisture; 3) Ninth-week oil from seed incubated in a water-saturated atmosphere; 4) Standard solution: (*e*) tripalmitin, (*f*) oleic acid, (*g*) 1,3-dipalmitin, (*h*) monopalmitin; 5) Standard solution: (*a*) trivernolin, (*b*) vernolic acid, (*c*) 1,3-divernolin, (*d*) (+) *threo*-12,13-dihydroxyoleic acid.

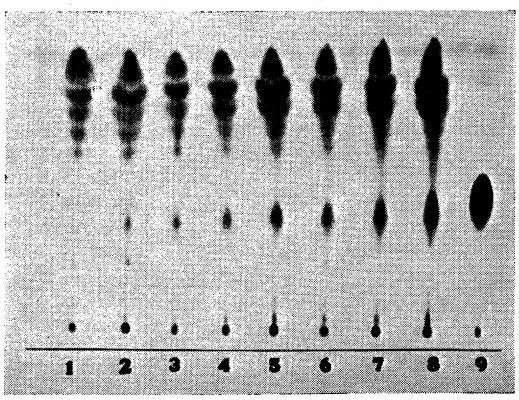

FIG. 5. Development of dihydroxyoleic acid in oil samples from seed incubated in a water-saturated atmosphere. 1–8) The second-week through the ninth-week oil samples; 9) (+) *threo*-12,13-dihydroxyoleic acid.

is shown also in Figure 4. Small amounts of nonepoxidized triglyceride and partially epoxidized triglyceride with one vernolate radical, partially epoxidized triglyceride with two vernolate radicals, nonepoxidized fatty acid, vernolic acid, nonepoxidized diglyceride, partially epoxidized diglyceride, divernolin, and dihydroxyoleic acid can be seen in this chromatogram. In addition, a trace amount of trivernolin may be present in the tail end of the spot representing nonepoxidized fatty acid.

The development and increase in amount of dihydroxyoleic acid with time of incubation can be seen in Figure 5.

The enzyme system in *Euphorbia lagascae* seed was not as active as that in *Vernonia anthelmintica* seed. Although the TLC studies showed that dihydroxy was formed, apparently by conversion of epoxy, no dihydroxyoleic acid was isolated. The high iodine values obtained in our *Vernonia* enzyme studies (1) were explained by the isolation of (+) *threo*-12,13-dihydroxyoleic acid, since Gunstone (7) had reported that some unsaturated hydroxy compounds gave abnormally high values. The relatively low iodine values of the oils obtained from incubated *Euphorbia* seed was further indication that the enzymes were not as active and that the conversion to dihydroxy did not proceed as far in *Euphorbia* as in *Vernonia*. However, the formation of dihydroxyoleic acid indicated the presence of a hydrating principle in *Euphorbia lagascae* seed similar to the one in *Vernonia anthelmintica* seed.

ACKNOWLEDGMENT

Analyses and technical assistance by M. E. Snook; drawings by A. J. Menna; photographic work by M. C. Audsley.

REFERENCES

1. Scott, W. E., C. F. Krewson, F. E. Luddy and R. W. Riemenschneider, JAOCS *40*, 587–589 (1963).
2. Miwa, T. K., F. R. Earle, G. C. Miwa, and I. A. Wolff, Ibid. *40*, 225–229 (1963).
3. Krewson, C. F., and W. E. Scott, "Seed Oil from *Euphorbia lagascae* Spreng. Abundant in Epoxyoleic Acid," Ibid., in preparation.
4. Krewson, C. F., J. S. Ard and R. W. Riemenschneider, Ibid., *39*, 334–340 (1962).
5. Krewson, C. F., and W. E. Scott, Ibid., *41*, 422–426 (1964).
6. Kleiman, R., C. R. Smith, Jr., S. G. Yates and Q. Jones, Ibid., *42*, 169–172 (1965).
7. Gunstone, F. D., J. Chem. Soc. 1274–1278 (1952).

[Received November 5, 1965]