Euphorbia lagascae Spreng., an Abundant Source of Epoxyoleic Acid; Seed Extraction and Oil Composition

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Abstract

Euphorbia lagascae Sprengel seed contains an unique epoxy-bearing oil (58–62%, 12,13-epoxyoleic acid) in high quantity (42–50%) with possible industrial potential. Oil from domestically grown seed was equal in quality and quantity to that obtained from Spain. It was readily and completely extracted at room temperatures without lipolysis using low solvent (petroleum naphtha) to seed ratios. The extracted crude oil was about equal in quality to that obtained by cold-pressing. Analytical data on the seed oil are compared with those previously reported for the oil of *Vernonia anthelmintica* (L.) Willd. (ironweed) seed.

Introduction

THE UNUSUAL EPOXY MAKEUP of the oil in *Euphorbia lagascae* Spreng. seed was recently discovered by Kleiman, Smith, Yates and Jones (5). The high oil content of *E. lagascae* seed coupled with an abundance of vernolic (12,13-epoxyoleic) acid in the oil provides excellent reasons for extended agronomic and chemical evaluation studies. *E. lagascae* seed differs from good quality *Vernonia anthelmintica* (L.) Willd. (ironweed) seed in that the vernolic acid is present in its oil chiefly as randomly or restricted randomly distributed glycerides (5). In *V. anthelmintica* the vernolic acid is present principally in the form of a single triglyceride, trivernolin (6).

Evidence of the widespread occurrence of epoxy fatty acids in plant seed oils is accumulating (6). The most important epoxy-bearing seed found to date has been that of *V. anthelmintica* which was discovered by Gunstone (3) in 1955. Vernolic acid was first isolated by Smith et al. (15) in 1959. Extended developmental studies at this laboratory (6–8,12,14) followed. There appears to be no information in the literature on the chemistry of *E. lagascae* prior to the report of Kleiman et al. (5). In fact, little has been written on this particular species of the *Euphorbia* genus, Euphorbiacae (Spurge) family. Lawrence (9) estimates that the genus contains over 1600 species. Sprengel (16) is responsible for the present name, *Euphorbia lagascae* Sprengel, which first appeared in 1821. He described the plant and referred to a former name, *Euphorbia terracina* Lag., and also mentioned its similarity to *Euphorbia obsoura* Loisel. He stated that its origin was unknown. Later, in 1826, Sprengel (17) classified *E. lagascae* in his "Systema Vegetabilium." Nyman (10) also used the synonym, *E. terracina* Lag., and mentioned Southern Spain as its habitat. Willkomm (20) confirmed this as the chief location. The following information on *E. lagascae* was supplied by Professor Regueral of Madrid in a recent communication (11).

In Spain, where *E. lagascae* is said to be native to that country, it occurs in the arid southeast and in all of the coastal region of Cadiz Provence. It appears in cultivated ground around towns generally in fallow land, rich in nitrogen. It does not grow in siliceous nor poor soils but needs limey sites of a saline nature. *E. lagascae* flowers in early spring, March to April, fruits in April and May depending upon climate such as the warm Mediterranean areas or on the steep slopes of flat-topped rocky hills. The plant is a coarse annual with smooth light green stems about 12–18 in. tall. The leaves are ovate-linear below and narrowly lanceolate above, obtuse, entirely or somewhat undulate. The leaves are in umbels and subtending. The flowers are subcordate, ovate-lanceolate to triangle-lanceolate. The umbels radiate, three repeatedly bifed. The capsule is ovate and acutely ribbed, lightly reticulated-veined and scarlet in varied shades. The seeds are cimereous, black marked or speckled, ovate, truncate and subcompressed. The caruncle is dish-like and obliquely emarginate. Figure 1 is a photograph of a drawing taken from Boissier (1). Figure 2 shows *E. lagascae* growing at Glenn Dale, Md. (photograph by J. J. Higgins), and Figure 3 is a photograph of seeds magnified ten times (photograph by M. C. Audsley).

FIG. 1. *Euphorbia lagascae* Spreng.

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TABLE I

Analytical Data on *Euphorbia lagascae* Seed and Oil

Source of seed Identity No.	Seed: Wt/ 1,000 g	Seed: Oil content % dry basis (d.b.)	Seed: Protein content N × 6.25, % (d.b.)	Oil properties: Epoxy-oleic acid, calc'd from oxirane oxygen %	Oil properties: FFA (as epoxy-oleic acid) %	Oil properties: Iodine value	Oil properties: Refractive index n_D^{40}	Composition of methyl esters, % by wt. (GLC): Carbon chain length : no. double bonds 14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	Other components
Spain [a] NU Sp 43571	7.4	42	26	58	.....	88	1.4676	.....	4	2	20	12	0.5	.....	0.8	60[b]
Spain NU Sp 44776	10.1	50	21	62	0.68	92	1.4675	0.87	3.86	1.41	18.6	8.18	0.23	0.30	....	66.5[c]
Glenn Dale, Md., 1964 from NU Sp 43571	9.8	48	22	60	0.46	88	.....	1.22	4.28	1.42	20.1	9.15	.....	.....	....	63.8[d]

[a] Data from (5).
[b] 57% Vernolate.
[c] 63.7% Vernolate.
[d] 62.0% Vernolate.

According to White (19), advanced evaluation of *E. lagascae* was planned for the 1965 growing season with cultural experiments to be conducted at Chico, Calif.; Manhattan, Kan.; and Glenn Dale, Md. Also, observational or modest increase plantings were scheduled at several other locations.

This report presents information on the extraction, composition and refining of *E. lagascae* seed oil. The potentials of this oil possibly overshadow those of the rich epoxy-bearing *V. anthelmintica* seed oil because domestically-grown *E. lagascae* seed contains almost twice the quantity of oil. *E. lagascae* seed meal prepared in this investigation is undergoing feeding experiments at the Pharmacology Laboratory of the Western Regional Research Laboratory. Other reports on *E. lagascae* from this laboratory include one on a comparative evaluation of *Euphorbia* and *Vernonia* products with respect to their plasticizer-stabilizer properties in poly(vinyl chloride) formulations (8) and another on the activity of *E. lagascae* seed enzymes (13).

Materials and Methods

The *E. lagascae* seed (Nu Sp 44776) used for the major portion of this investigation was of Spanish origin collected from wild plants under Public Law 480 funds as arranged for by Quentin Jones of the Crops Research Division of this Department. A 200 g sample of domestically grown *E. lagascae* from a 1964 planting at Glenn Dale, Md., was supplied by G. A. White of the same Division.

Analytical data on *E. lagascae* seed oil were obtained by AOCS procedures; the Durbetaki (2) method as modified previously (6) was used to determine oxirane oxygen percentage; gas-liquid chromatographic analyses of methyl esters prepared from *E. lagascae* seed oil employed the procedure of Herb and Magidman using techniques previously described (4). The method (7) used for the estimation of the oil content of *E. lagascae* seed was the same as for *V. anthelmintica* seed and involved a rapid extraction procedure with petroleum naphtha (P.N.) (bp 35–59C) as solvent. The procedure prevents lipolysis and gives oils with low free fatty acid (FFA) content. The refining of *E. lagascae* seed oil required the same low temperature crystallization technique as *V. anthelmintica* (7).

TABLE II

Characteristics of *E. Lagascae* Oil

Specific gravity, 25C	0.9494
Optical rotation $[\alpha]_D^{25}$ (plus)	1.76[a]
Gardner color no.	8–9
Saponification value	188.8
Unsaponifiable material %	0.71
Hydroxyl value	10.4[b]
Reichert-Meisal value	0.42
Polenski value	0.21

[a] n-hexane.
[b] 4 hr acetylation instead of usual 1 hr.

Results and Discussion

A. Compositional Studies

Three *E. lagascae* seed samples have been examined to date: the first, NU Sp 43571 (42% oil) from Spain was analyzed at the Northern Regional Research Laboratory (5) and is included in Table I for comparative purposes; the second, NU Sp 44776 (50% oil) represents a later seed sample from Spain; the third (48% oil) is a domestic seed sample grown in 1964 at Glenn Dale, Md., from NU Sp 43571. The rapid extraction technique for oil which is considered quantitative gave the percentages 50 and 48%. A figure of 44.5% oil for NU Sp 44776 obtained by exhaustive (20 hr) Soxhlet extraction did not compare too favorably with the figure of 50% obtained by the rapid technique. The FFA in the extracted oil after a 4 hr soxhlet extraction period was 3.43%. This compares with 0.68% obtained in the Waring Blendor rapid method, and is indicative of the degree of lipolysis which occurred with grinding and slower extraction. In the rapid technique *E. lagascae* seed oil was completely extracted with only three 400 ml

TABLE III

Solvent Extraction of *E. Lagascae* Seed[a]
1 kg Seed Extraction by Rapid Method with PN at Room Temperature

Extract No.	Quantity		FFA as epoxy-oleic acid	Oxirane oxygen	Iodine value (Wijs)
	g	%	%	%	
1	406.0	42.5	0.97	3.34	91.7
2	51.9	5.43	2.24	3.16	92.7
3	2.32	0.24	11.19	2.62	92.7
4	0.65	0.01	7.80	0.34	.... [b]
Total oil	460.9	48.2			
Meal [c]	509.0	50.9			

[a] 4.5% moisture, 50.0% oil
[b] Insufficient quantity for analysis
[c] 43.7% protein, 11.1% ash, 0.53% oil

TABLE IV

Qualities of Solids Obtained

Solvent	Product		
	Wt, g	%	Description
PN.	93.4	48.9	Yellow oil
Diethyl ether	0.49	0.24	Brown resin
Methanol	8.50	4.45	Brown solid
Chloroform	0.03	0.01	Yellow resin

P.N. extractions of a 50.0 g sample whereas eight (7) were required to completely extract *V. anthelmintica* seed oil.

A comparison of the *E. lagascae* data (Table I) with that previously reported (6, 7, 18, 19) for *V. anthelmintica* shows that the seed of the former contained almost twice as much oil (42–50%) as the latter (25–30%). The 58–62% epoxyoleic acid in *E. lagascae* seed oil apparently has random or restricted random distribution in the glycerides (5). In *V. anthelmintica* the 65–75% of the same acid is present principally as a single triglyceride, trivernolin (7). *E. lagascae* seed oil contained considerably more oleic acid (18–20%) than *V. anthelmintica* seed oil (2%). However, both oils contained about the same quantities of linoleic acid (8–12%) and total saturated acids (3–6%). A noteworthy difference in seed oil composition between these two plants was the relatively high quantity of unsaponifiable material in *V. anthelmintica* (7–8%) compared with less than 1% in *E. lagascae*. This is probably the reason for the difference in iodine values of the two oils.

Oil extracted from *E. lagascae* (NU Sp 44776) by P.N. using the Waring Blendor (7) had the following characteristics as shown in Table II in addition to those listed in Table I. The oil has a yellow color, is liquid at room temperatures but slowly gels at 4C.

B. Extraction of E. lagascae Seed

E. lagascae seed (NU Sp 44776, moisture 4.95%) was rapidly extracted in a Waring Blendor using P.N. at room temperature. A kilogram sample of seed was divided into ten 100 g portions and each macerated for 2–3 min with 400 ml of P.N. in the Blendor. Each mixture was poured into the same 10 in. Buchner funnel equipped with a No. K-5 (Republic Seitz Filter Corp., Newark, N. J.) filter pad. After each pulverization the container was washed out with an additional 400 ml of P.N. and the washings poured over the meal on the filter plate. The extracts and washings of all 10 portions became the miscella (8 l) designated as the first extract (Table III). The meal was divided into 10 approximately equal portions and the procedure repeated for the second extract. A third and fourth extract were made in similar fashion. The oil was obtained from each miscella by removal of the solvent at reduced pressures using a rotating thin-film evaporator. Data on each fraction are presented in Table III. Of the total oil extracted 88.1% was found in the first extract and 11.3% in the second to yield 99.4% in the first two extracts. The data obtained indicate that *E. lagascae* was readily extracted with P.N. at room temperatures with little or no lipolysis when using a solvent ratio of about 1.5 to 2 gal per pound of seed and a well-pulverized seed.

Selective solvent extractions were made of a 200 g sample of NU Sp 44776 using the Waring Blendor rapid technique followed by exhaustive Soxhlet (20 hr) extractions. PN was used first, followed by diethyl ether, absolute methanol and chloroform. The quantities of solids obtained from each extract are shown in Table IV.

TABLE V

E. lagascae Oils—Comparative Evaluation of Refining Techniques (Solvent Extraction vs. Cold Pressing)

		Crude oil by cold (4C) PN extractions	Refined oil	Oil by cold pressing	Oil by cold PN extn. of press-cake
Yield (d.b.)	%	50.0	42.3	25.3	22.7
Oxirane oxygen	%	3.33	3.40	3.39	3.21
FFA (as epoxyoleic)	%	0.68	0.18	0.60	1.4
Iodine value (Wijs)		91.8	90.7	86.4	88.3
Unsaponifiable matter	%	0.71	0.35	.....	.....
Color (Gardner)		9	1	5	10

C. E. lagascae Seed Oil by Cold-Pressing

A 55.9 g sample of *E. lagascae* seed (4.45% moisture) was subjected to a pressure of 4000 psi in a

FIG. 2. *Euphorbia lagascae* Spreng. growing at Glenn Dale, Md.

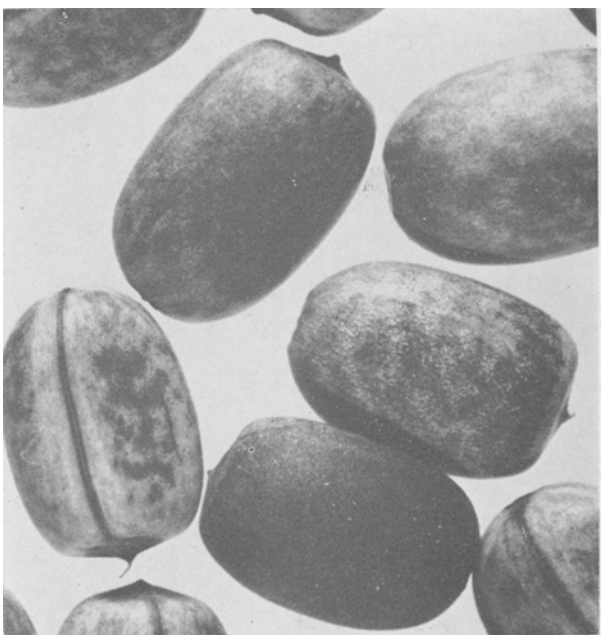

FIG. 3. *Euphorbia lagascae* Spreng. seed (10X magnification).

Carver press to obtain 13.5 g (25.3%) of oil. The press-cake was then extracted by the rapid technique (7) and yielded an additional 12.1 g (22.7%) of oil. Comparative analytical data are presented in Table V. This table also includes information on crude and refined *E. lagascae* seed oil obtained from unpressed seed by the same procedure.

No water-insoluble volatile oil was obtained from the steam distillation of 400 g of *E. lagascae* seed previously ground in water in a Waring Blendor. The distillate which had a nutty odor was repeatedly cohobated but no insoluble material could be obtained on cooling the final distillate to about 4C.

D. Trivernolin from E. lagascae

A 51.0 g sample of *E. lagascae* seed oil prepared from sample NU Sp 44776 gave a yield of 12.0 g (23.5%) trivernolin by the procedure previously used (6,7) to obtain trivernolin from *V. anthelmintica.* This figure is in reasonably close agreement with the value of 20% found by Klieman and co-workers (5) in NU Sp 43571 using a similar procedure. The low value also helps confirm their indication that in *E. lagascae* seed oil the epoxyoleic acid is randomly or restricted randomly distributed. This is in sharp contrast to epoxyoleic acid occurrence in *V. anthelmintica* where it is present principally as a single glyceride, trivernolin.

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