The monoglycerides of each acid contains approximately 10% of the 2-isomer.

The 2-monoolein area includes the 1-monostearin peak while the 2-monolinolein peak is masked by the 1-monoolein peak. Monoglyceride TMS derivatives have been separated according to degree of unsaturation on thin layer plates. However, this technique is not perfected and is under further investigation. With the use of this additional step one can quantitatively determine the composition of any monoglyceride mixture, including the conen of the 1- and 2-isomers of each component.

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REFERENCES

1. McInnes, A. G., N. H. Tattrie and M. Kates, JAOCS *37*, 7 (1960).
2. Huebner, V. R., *Ibid. 36*, 262 (1959).
3. Martin, J. B., J. Am. Chem. Soc. *75*, 583 (1953).
4. Langer, S. H., P. Pantages and I. Wender, Chem. Ind. (London) 1664 (1958).
5. Shaw, R. O. D., Anal. Chem. *35*, 1580 (1963).
6. Hedgley, E. J., and W. G. Overend, Chem. Ind. (London) 379 (1960).
7. Sweeley, C. C., R. Bentley, M. Makita and W. W. Wells, J. Am. Chem. Soc. *85*, 2497 (1963).
8. Luukkainen, T., W. J. A. VanderHeuvel, E. O. A. Haati and E. C. Horning, Biochim. Biophys Acta *52*, 599 (1961).
9. Wells, W. W., and M. Makita, Anal. Biochem. *4*, 204 (1962.)
10. Makita, M., and W. W. Wells, *Ibid. 5*, 523 (1963).
11. Litchfield, C., R. Rieser and A. F. Isbell, JAOCS *40*, 302 (1963).
12. Stahl, E., Chemiker Ztg. *82*, 323 (1958).
13. Stahl, E., Pharmazie *11*, 633, (1956).
14. Youngs, C. G., A. Epp, B. M. Craig and R. H. Sallons, JAOCS *34*, 107 (1957).
15. Mattson, F. H., and R. A. Volpenhein, J. Lipid Res. *3*, 281 (1962).
16. Hartman, L., J. Chem. Soc. (London) 3572 (1957).
17. Analytical Engineering Laboratories Inc., Hamden, Conn.
18. Litchfield, C., M. Farquhar and R. Reiser, JAOCS *41*, 588 (1964).

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Helichrysum Seed Oil. I. Separation and Characterization of Individual Acids

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Abstract

Helichrysum bracteatum (family Compositae) seed oil contains a complex array of unusual fatty acids in addition to the usual palmitic, stearic, oleic, and linoleic acids. Its unusual constituents include 9.5% crepenynic acid; 14.0% epoxy acids, mainly coronaric; 4.4% *cis,trans*-hydroxy conjugated dienoic acids; and 7.2% of a previously unknown hydroxyacetylenic acid for which the name helenynolic acid is proposed. A method for determining helenynolic acid in the presence of hydroxy-conjugated dienes is described.

Introduction

Helichrysum bracteatum or strawflower is an annual originally from Australia. It is widely cultivated in the United States for its bloom, since the flower heads, when dried, hold their color for months.

The unusual nature of the oil was first noted in this laboratory during a routine gas-liquid chromatographic (GLC) analysis of the methyl esters. No linolenic acid was found although oil from a different accession of seed had been reported to contain 32.0% of this acid as determined by alkali isomerization (1). An unusual component appeared with equivalent chain lengths (ECL) of 18.1 on an Apiezon L column and 20.7 on a LAC-2-R446 column (2) and was tentatively identified as methyl crepenynate (3). A Durbetaki titration (4) of the oil gave a hydrogen bromide equivalent (HBE) (5) of 18.4, whereas GLC analysis of the methyl esters indicated a much lower quantity of epoxy acids to be present. An IR spectrum of the oil contained a noticeable hydroxyl peak, but no other peculiar feature that seemed significant. A UV absorption maximum at 229 mμ (6) indicated conjugated enyne. Thin-layer chromatography (TLC) of the methyl esters revealed the presence of three monohydroxy acids and at least one epoxy acid.

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This paper presents the isolation and purification of the individual component fatty acids of *Helichrysum* oil, and the characterization of some of the unusual acids present.

Experimental Procedures and Data

Methods

TLC was performed on glass plates coated with Silica Gel G (according to Stahl) with hexane, diethyl ether, acetic acid (70:30:1 or 80:20:1) as the solvent. Spots were detected by spraying with 50% sulfuric acid and heating at 120C.

GLC analyses were carried out as described by Miwa et al. (2).

Countercurrent distribution (CCD) studies utilized a 200-tube Craig-Post apparatus and the acetonitrile-hexane solvent system (7).

Partition chromatography of *Helichrysum* free acids was carried out essentially as described by Frankel et al. (8,9) using a 2.0% methanol in benzene as the eluting solvent. Our separations were not comparable to those of Frankel because of the large samples used; however, it was possible to obtain a hydroxy acid concentrate as described later in this paper.

Periodate-permanganate oxidations were as described by von Rudloff (10).

UV spectra were determined with a Beckman DK-2A spectrophotometer. IR spectra were determined on a Perkin-Elmer Model 137 instrument, using 1% solutions in carbon tetrachloride.

Oil Extraction. Oil was obtained from the ground seed of *Helichrysum bracteatum* var. *monstrosum* by Soxhlet extraction with petroleum ether (bp 30–60C). Solvent was removed *in vacuo* with a rotary evaporator. The yield of oil was 26.6%. It showed a rather broad maximum at 229.7 mμ, $E^{1\%}_{1\,cm}$ 80.5, in cyclohexane. In the IR, a hydroxyl peak (3450 cm^{-1}) was the only unusual feature. The oil had an HBE of 18.4 and contained 0.7% free fatty acid (calculated

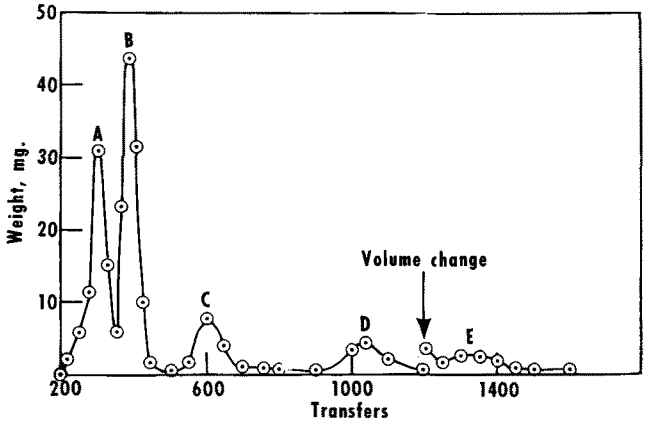

FIG. 1. Countercurrent distribution of *Helichrysum* methyl esters. Transfers 1–1200 each contained 10 ml of upper phase; transfers after 1200 contained 40 ml of upper phase.

as oleic). For other analytical data on the crude oil see reference 1.

Preparation of Methyl Esters. Helichrysum seed oil (14.92 g) was saponified by refluxing it with 1 *N* potassium hydroxide under nitrogen for 1.5 hr. The saponification mixture was extracted with ethyl ether, acidified with 3 *N* hydrochloric acid, and extracted again with ether. A yield of 0.82 g of unsaponifiable material and 10.79 g of fatty acids was obtained. The HBE of the unsaponifiables was only 0.8.

A portion of the fatty acid mixture was esterified by refluxing for 1 hr with 1% sulfuric acid in methanol. The esters were isolated by diluting the reaction mixture with water and extracting with ethyl ether. Another portion of free acids was esterified in ether solution by the addition of diazomethane.

Methyl esters were also prepared by alcoholysis of the crude oil with sodium methoxide catalyst; 10.15 g of oil was dissolved in 100 ml of methanol in which 0.4 g of sodium had been dissolved previously. This mixture was refluxed under nitrogen for 1 hr, acidified with acetic acid, diluted with an equal volume of water, and extracted with ether. A yield of 8.3 g of mixed esters was obtained.

CCD of Mixed Methyl Esters. A 7.86-g sample of mixed esters, obtained by alcoholysis, was subjected to CCD following the single withdrawal procedure. The sample was dissolved in 40 ml of upper phase and distributed equally among the first four tubes. After 1,000 transfers had been made with 10 ml of upper phase per transfer, the apparatus was set to deliver

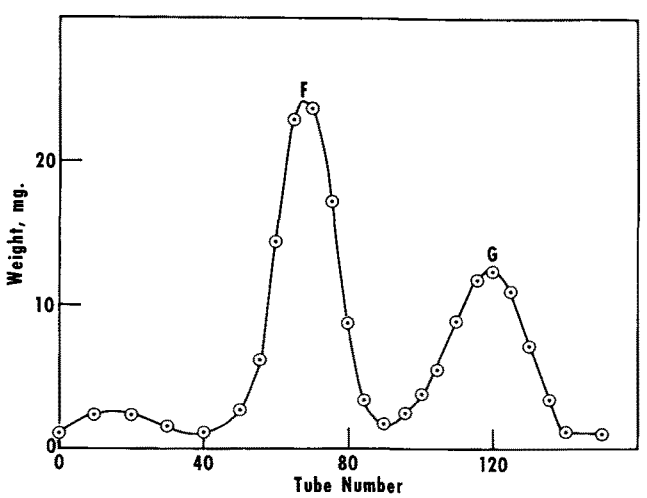

FIG. 2. Countercurrent distribution of hydroxy esters.

40 ml of upper phase, and the distribution was continued for another 650 transfers. The weight distribution is shown in Figure 1. Peak C amounted to 12.7% of the original sample and peak D was 14.0%.

The monohydroxy esters did not emerge from the CCD apparatus during this run. UV spectra of selected tubes revealed a conjugated diene (λ_{max} 233 mμ) to be concentrated in tubes 150–200 and a conjugated enyne (λ_{max} 228 mμ) in tubes 90–130. Considerable unsaponifiable material was found to be present in these tubes when examined by TLC.

Concentration of Hydroxy Acids by Column Chromatography. After removal of unsaponifiable material, mixed *Helichrysum* free acids could be chromatographed on silica gel to obtain a concentrate of the hydroxy acids. The acids were chromatographed in 1.5-g batches. The separation was followed by IR spectroscopy and appropriate fractions were combined. The concentrate was further enriched in hydroxy acids by chromatographing it a second time. A total of 2.67 g of concentrate was obtained from 14.21 g of the original free acid mixture. The concentrate was then esterified with diazomethane and the resulting esters were chromatographed on silica gel using hexane-ethyl ether (80:20) as the eluting solvent. After combining fractions rich in hydroxyl as determined by the presence of a peak at 3600 cm^{-1} in the IR, the concentrate amounted to 1.17 g.

CCD of Hydroxy Esters. The hydroxy ester concentrate, 1.17 g, was subjected to a 600 transfer CCD similar to the previous one except that 40-ml transfers of upper phase were used throughout. The wt distribution (fundamental procedure) is shown in Figure 2. Peak F contained 4.0% (0.57 g) and peak G contained 2.5% (0.35 g) of the total methyl esters derived from the original oil. These must be regarded as minimum percentages for the respective components, as losses undoubtedly occurred during the concentration procedure.

TLC and GLC Studies. Mixed methyl esters prepared by each of the methods discussed previously were examined by TLC (Fig. 3). Diazomethane-prepared esters (sample 3) gave three spots with R_f values comparable to monohydroxy esters, and one major spot with R_f slightly less than that of 9,10-epoxystearate. TLC analysis of esters prepared with methanolic sulfuric acid showed the absence of epoxy acids as well as two of the monohydroxy acids; however, two new spots were evident having R_f values of 0.71 and 0.78.

The peaks obtained by CCD were also investigated by TLC (see Fig. 4). Sample 3 (Fig. 3) and sample 3 (Fig. 4) were both prepared by diazomethane esterification of *Helichrysum* free acids; removal of unsaponifiables was complete in second but not in the first sample. The more polar solvent system used (hexane : ethyl ether-acetic acid, 70 : 30 : 1) gave much better resolution of the monohydroxy esters.

In the GLC analyses of the methyl esters summarized in Table I, monohydroxy esters are not listed because the chromatograms were not continued long enough for these esters to emerge from the column. A concurrent analysis of an Apiezon-L column of the esters prepared by alcoholysis gave results similar to those in Table I, except that there were two peaks in the epoxy region (ECL 19.2 and 19.4) which amounted to 9.1% and 2.2%, respectively.

Characterization of CCD Peaks

Peak A. Peak A (Fig. 1) was shown by GLC to be

a mixture of palmitate (42.0%), stearate (15.7%), and oleate (41.0%).

Peak B. Peak B was 98.5% methyl linoleate by GLC.

Peak C. It contained methyl crepenynate in 99.5% purity by GLC and gave only a single spot when examined by TLC (Fig. 4). The IR spectrum was identical to methyl crepenynate obtained from *Crepis foetida* seed oil (11).

This ester was hydroxylated by essentially the same procedure used by Gunstone and Russell (12) in the identification of ximenynic acid. The material (0.111 g) in peak C was warmed with a mixture of 0.5 ml of 90% formic acid and 0.1 ml of 30% hydrogen peroxide. The mixture was held at 40C for 2 hr. Solvent was removed under reduced pressure, 10 ml of 3 *N* potassium hydroxide added to the residue, and the mixture refluxed for 1 hr. The solution was acidified with dilute hydrochloric acid, extracted with ether, and dried over anhydrous sodium sulfate. A light yellow oil (70.8 mg) was obtained after removal of solvent. Two crystallizations of this oil from chloroform-hexane yielded 14.8 mg of a dihydroxyacetylenic acid, up 71–72C. Hydrogenation of the acid in ethanol (Adams' catalyst) yielded *threo*-9,10-dihydroxyoctadecanoic acid, mp 93–94C. The melting point was undepressed upon mixture with authentic *threo*-9,10-dihydroxyoctadecanoic acid, mp 93–94C. On admixture with *threo*-12,13-dihydroxyoctadecanoic acid (mp 95–96C) the melting point was lowered to 83–91C.

To verify the position of the triple bond, 40.0 mg of C was oxidized with 25 ml of periodate-permanganate reagent, 15 ml of tertiary butanol, and 0.5 g of potassium carbonate. GLC analysis of the free acids showed essentially pure hexanoic. Methyl esters were prepared, and GLC analysis of this revealed methyl hexanoate and dimethyl azelate along with some minor short-chain contaminants. Thus structure I (Table II) was verified.

Peak D. The material in peak D was shown to be an epoxyoleate (99.3%) by GLC analysis. TLC analysis revealed one major spot with a minor overlapping one of slightly higher R_f (Fig. 4). D (0.192 g) was refluxed with 10 ml of glacial acetic acid for 3 hr under a nitrogen atmosphere (13). The bulk of acetic acid was removed by evaporation under reduced pressure, and the residue was refluxed for 2 hr with 15 ml of 3 *N* potassium hydroxide. The mixture was acidified with 3 *N* HCl and 79 mg of a yellow oil was recovered by ether extraction. The oil was crystallized twice from chloroform-hexane; a yield of 17 mg of a dihydroxy acid (mp 58–62C) was obtained. The dihydroxy acid (14 mg) was hydrogenated with a palladium-charcoal catalyst in glacial acetic acid. The hydrogen uptake was 1.01 moles and the resulting dihydroxystearic acid had a melting point of 93–93.5C. On admixture with authentic *threo*-9,10-dihydroxyoctadecanoic acid (mp 93–94C) the melting point was 92.5–94C. Mixture with *threo*-12,13-dihydroxyoctadecanoic acid lowered the mp to 84–92C.

A portion of the dihydroxyoleate was oxidized with periodate-permanganate reagent as described earlier for methyl crepenynate. GLC analysis of the oxidation products (both as free acids and as methyl esters) showed essentially only hexanoic and azelaic acids. These data are consistent only with coronaric acid (structure II, Table II).

Peak E. This peak contained material having an ECL of 23.7 on R-446 and 19.4 on Apiezon-L with a purity of 96.2%. It was not characterized further.

FIG. 3. TLC of *Helichrysum* methyl esters: 1) Standard mixture for comparison in order of increasing R_f: ricineleidate, 9,10-epoxystearate, 12-ketostearate, palmitate. 2) Esters from sodium methoxide alcoholysis. 3) Esters prepared with diazomethane. 4) Esters prepared using 1% sulfuric acid in methanol; spots having R_f values of 0.71 and 0.78 are unusual oxygenated compounds. The developing solvent was hexane:ethyl ether:acetic acid (80:20:1). Spots were reproduced by tracing.

Peak F. It showed a single spot when examined by TLC (Fig. 4). The material had the following spectral characteristics: IR showed peaks at 3600 cm^{-1} (−OH), 2210 cm^{-1} (C≡C), 1625 cm^{-1} (C=C), and 956 cm^{-1} (*trans* C=C). UV had λ_{max} 227.8 mμ ($E^{1\%}_{1cm}$ 570) in ethanol with an inflection at 235 mμ. Material from peak F was not reactive under conditions of the Durbetaki titration. When GLC examination of this material was attempted, it decomposed into a number of unidentified fragments. This decomposition was more pronounced on an Apiezon-L column, probably because of the higher temp used.

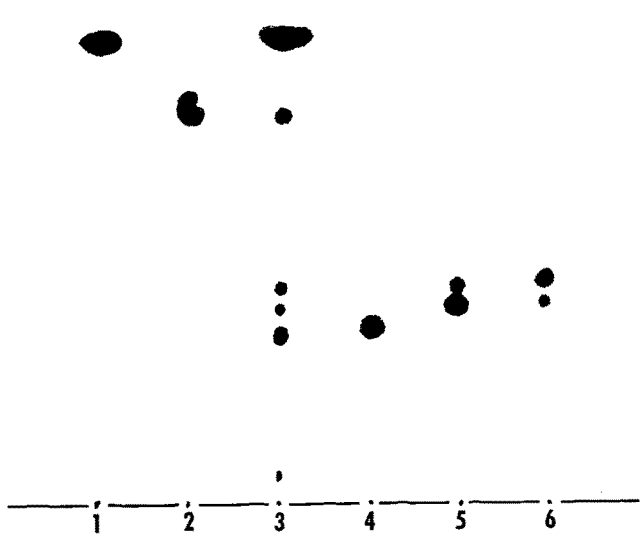

FIG. 4. TLC examination of countercurrent distribution peaks: 1) Peak C, methyl crepenynate. 2) Peak D, epoxy acid(s). 3) Mixed *Helichrysum* methyl esters, free of unsaponifiables; spot of R_f 0.06 is probably a dihydroxy ester. 4) Peak F, conjugated enynol. 5) and 6) Tubes 110 and 130 from peak G, conjugated dienols. The developing solvent was hexane:ethyl ether:acetic acid (70:30:1). Spots were reproduced by tracing.

TABLE I

GLC Analyses of *Helichrysum* Methyl Esters
(Results expressed as area percent)

Component	ECL[a]	Method of preparation		
		Methanol-sodium methoxide	Diazomethane	Methanol, sulfuric acid
Palmitate	16.0	12.2	12.7	12.6
Stearate	18.0	4.6	4.3	4.2
Oleate	18.4	11.9	11.5	12.0
Linoleate	19.0	48.9	54.0	53.8
Crepenynate	20.8	13.0	9.6	8.8
Epoxyoleate	23.1	5.8	4.3	.....
Unknown	Various	3.6	3.6	8.6

[a] As determined on Resoflex column. For explanation of ECL, see reference 2.

Evidence for structure III (Table II) will be presented elsewhere (14).

Peak G. TLC examination revealed a mixture of hydroxy acids in peak G (Fig. 4). This mixture had a UV maximum at 233 mμ. IR showed peaks at 3600 cm^{-1} (−OH) and the peaks of nearly equal intensity at 980 and 950 cm^{-1} indicated a *cis,trans*-conjugated diene (15).

Method for Determining Enynol in the Presence of Dienol. Material from peak F (conjugated enyne) was not dehydrated by refluxing for 1.5 hr with 0.1 *N* methanolic HCl and retained essentially all the enyne chromophore; no significant increase in absorbance was noted in the conjugated triene region. Under these conditions conjugated dienols similar to dimorphecolic acid are dehydrated to trienes (16) as were the dienols from peak G. A sample of *Helichrysum* oil was refluxed with 0.1 *N* methanolic HCl for 1.5 hr. UV spectra of the oil before and after the acidic methanol treatment are shown in Figure 5. Assuming 100% dehydration of the dienols to *trans,trans,trans*-conjugated trienes and using the value ε 54,900 for λ_{max} 269 mμ (17), it was calculated that a minimum of 3.5% triene formed. Thus *Helichrysum* oil contains at least 3.5% of α-hydroxydienoic acids. It was also possible to calculate a maximum amount of enyne present (10.8%) if the value was used of $E^{1\%}_{1cm}$ quoted earlier for pure F. This percentage is high, due to interfering absorptions (Fig. 5).

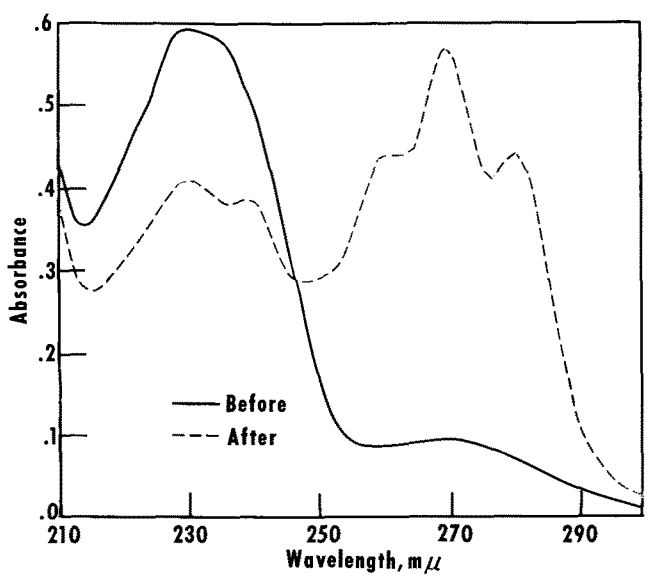

FIG. 5. Ultraviolet spectra of *Helichrysum* oil before and after treatment with refluxing 0.1 *N* methanolic HCl.

TABLE II

Structures (Confirmed and/or Tentative) of the Unusual Fatty Acids in *Helichrysum bracteatum* Seed Oil

I	$CH_3(CH_2)_4C{\equiv}CCH_2CH{=}CH(CH_2)_7CO_2H$ (*cis*)
II	$CH_3(CH_2)_4CH{=}CHCH_2CH{-}CH(CH_2)_7CO_2H$ (*cis*, O)
III	$CH_3(CH_2)_4C{\equiv}CCH{=}CHCH(OH)(CH_2)_7CO_2H$ (*trans*)
IV	$CH_3(CH_2)_4CH{=}CHCH{=}CHCH(OH)(CH_2)_7CO_2H$
V	$CH_3(CH_2)_4CH(OH)CH{=}CHCH{=}CH(CH_2)_7CO_2H$

Discussion

Helichrysum bracteatum seed oil contains crepenynic acid (I) which was first reported to occur in *Crepis foetida* seed oil by Mikolajczak et al. (3). Up to 14% epoxy acids are also present, the major portion of which has been characterized as coronaric acid (II) originally found in *Chrysanthemum coronarium* seed oil (18). The conjugated *cis,trans* dienols may have structures IV and V. These have been found as constituents of *Tragopogon porrifolius* seed oil (19), and evidence for their wide occurrence in seed oils of the Compositae has been presented by Morris et al. (20). The seed oil of another composite, *Dimorphotheca aurantiaca* (= *D. sinuata*), is known to contain ca. 59% dimorphecolic acid (21) as determined by UV spectral analysis. Dimorphecolic acid is the *trans, trans* isomer of IV (22).

Helichrysum bracteatum seed oil also contains an unusual hydroxyacetylenic acid which was originally thought to be 8-hydroxyximenynic acid (23). Further investigation has shown that it is a new acid (14) and the name helenynolic acid is proposed. The observation that methyl helenynolate (tentative structure III), with a hydroxyl group α to unsaturation, decomposes under GLC conditions is consistent with earlier work by Morris et al. (24). They found that reduced linoleate hydroperoxides and methyl dimorphecolate decomposed similarly, and that decomposition was related to the column and flash heater temp used. Compounds with hydroxyl groups that were not α to unsaturation were stable under the same conditions. Part of the unknown peaks in Table I are thought to be fragments from methyl helenynolate.

With our data, the total percentages of fatty acids present in *Helichrysum* oil were calculated. Conjugated dienes with hydroxyl α to olefinic unsaturation consume hydrogen bromide rapidly in the Durbetaki titration (25). Since only 14.0% epoxy acids were isolated from CCD, it was assumed that 4.4% of dienols must be present to allow for the observed HBE of 18.4. CCD of the hydroxy acid concentrate showed dienes and enynes to occur in the ratio of about 1.00 to 1.62. This ratio allows for 7.2% helenynolic acid. These percentages were used for the oxygenated acids, and the GLC values (Table I, alcoholysis esters) for nonoxygenated acids were recalculated on this basis. Revised percentages are given in Table III. The reason for low percentages of epoxy

TABLE III

Composition of *Helichrysum bracteatum* Seed Oil
(Combined results from GLC and CCD data)

Component	Percent of oil	Component	Percent of oil
Palmitic	9.0	Epoxy	14.0
Stearic	3.3	Conjugated diene	4.4
Oleic	8.7	Conjugated enyne	7.2
Linoleic	35.8	Unknown acids	2.6
Crepenynic	9.5	Unsaponifiables	5.5

acids obtained by GLC is not clear. Reactions may have occurred between the epoxides and any of the reactive constituents not ordinarily found together with them under conditions of the GLC analysis. Losses occurring from decomposition or alteration of epoxy acids on a number of stationary phases have been investigated by Herb and co-workers (26).

Evidence for the presence of small amounts of a dihydroxy acid is shown in sample 3 of Figure 4. Formation of unusual oxygenated compounds, probably methyl ethers of the hydroxy acids, is suggested by TLC of esters prepared with methanolic sulfuric acid (sample 4, Fig. 3). These oxygenated materials are undergoing further study.

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REFERENCES

1. Earle, F. R., I. A. Wolff and Q. Jones, JAOCS *37*, 254–256 (1960).
2. Miwa, T. K., K. L. Mikolajczak, F. R. Earle and I. A. Wolff, Anal. Chem. *32*, 1739–1742 (1960).
3. Mikolajczak, K. L., C. R. Smith, Jr., M. O. Bagby and I. A. Wolff, J. Org. Chem. *29*, 318–322 (1964).
4. Durbetaki, A. J., Anal. Chem. *28*, 2000–2001 (1956).
5. Wilson, T. L., C. R. Smith, Jr., and K. L. Mikolajczak, JAOCS *38*, 696–699 (1961).
6. Ligthelm, S. P., H. M. Schwarz and M. M. von Holdt, J. Chem. Soc.—1088–1093 (1952).
7. Scholfield, C. R., J. Nowakowska, and H. J. Dutton, JAOCS *37*, 27–30 (1960).
8. Frankel, E. N., C. D. Evans, H. A. Moser, D. G. McConnell and J. C. Cowan, *Ibid. 38*, 130–137 (1961).
9. Frankel, E. N., D. G. McConnell and C. D. Evans, *Ibid. 39*, 297–301 (1962).
10. Rudloff, E. von, Can. J. Chem. *34*, 1413–1418 (1956).
11. Mikolajczak, K. L., Personal communication from this laboratory.
12. Gunstone, F. D., and W. C. Russell, J. Chem. Soc.—3782–3787 (1955).
13. Gunstone, F. D., *Ibid.*—1611–1616 (1955).
14. Powell, R. G., C. R. Smith, Jr., C. A. Glass and I. A. Wolff, J. Org. Chem., in press.
15. Tolberg, W. E., and D. H. Wheeler, JAOCS *35*, 385–388 (1958).
16. Smith, C. R., Jr., C. A. Glass and I. A. Wolff, U.S. Pat. 3,027,388 (1962).
17. Holman, R. T., W. O. Lundberg and T. Malkin, eds., "Progress in the Chemistry of Fats and Other Lipids," Vol. 4, p. 240, Pergamon Press, New York, 1957, p. 240.
18. Smith, C. R., Jr., M. O. Bagby, R. L. Lohmar, C. A. Glass and I. A. Wolff, J. Org. Chem. *25*, 218–222 (1960).
19. Chisholm, M. J., and C. Y. Hopkins, Can. J. Chem. *38*, 2500–2507 (1960).
20. Morris, L. J., R. T. Holman and K. Fontell, JAOCS *37*, 323–327 (1960).
21. Earle, F. R., K. L. Mikolajczak, I. A. Wolff and A. S. Barclay, *Ibid. 41*, 345–347 (1964).
22. Smith, C. R., Jr., T. L. Wilson, E. H. Melvin and I. A. Wolff, J. Am. Chem. Soc. *82*, 1417–1421 (1960).
23. Ligthelm, S. P., Chem. Ind. (London)—249–250 (1954).
24. Morris, L. J., R. T. Holman and K. Fontell, J. Lipid Res. *1*, 412–420 (1960).
25. Smith, C. R., Jr., M. C. Burnett, T. L. Wilson, R. L. Lohmar and I. A. Wolff, JAOCS *37*, 320–323 (1960).
26. Herb, S. F., P. Magidman and R. A. Barford, *Ibid. 41*, 222–224 (1964).

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Search for New Industrial Oils. XII. Fifty-eight Euphorbiaceae Oils, Including One Rich in Vernolic Acid[1]

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Abstract

Seed oil of *Euphorbia lagascae* Spreng. contains 57% of *cis*-12,13-epoxy-*cis*-9-octadecenoic (vernolic) acid. The amt of trivernolin in the glycerides of this species indicates random or restricted random distribution of the vernolic acid.

Seed from 57 additional species in the Euphorbiaceae were analyzed for oil and protein contents and also for fatty acid composition of the oils. Iodine values (I.V.) of the oils ranged from 87–221. Among these oils, samples were encountered with as much as 76% linolenic, 77% linoleic or 84% oleic acid.

Introduction

OF THE GLYCERIDE OILS now traded in quantity, two of the more unusual ones are from plants in the Euphorbiaceae (spurge family). Castor oil containing ricinoleic acid is obtained from *Ricinus communis* L., and tung oil containing eleostearic acid from several species of *Aleurites*. Literature reports of other unusual oils in the family include kamala oil from *Mallotus philippinenis* Muell. Arg. containing hydroxyeleostearic acid; croton oil from *Croton tiglium* L. possessing violent purgative properties; oil containing epoxy acids from *Cephalocroton* (1) ; and oils containing conjugated unsaturation from *Ricinodendron, Sapium,* and *Garcia*. Most of the oils from approx 65 species of Euphorbiaceae reported by Hilditch (10), Eckey (6), and in more recent literature contain only the common fatty acids in widely varying proportions.

In our continuing survey of seed oils, 58 species of Euphorbiaceae have been analyzed; 11 of these, including six in our earlier papers (3,4), have been reported in prior literature but without gas-liquid chromatographic (GLC) analyses. One species of the 58, *Euphorbia lagascae* Spreng., is unique in its high content of epoxyoleic acid.

The Euphorbiaceae include some 280 genera and 8,000 species (2), predominantly tropical but also widely distributed in temperature regions. The largest genera are *Euphorbia* (ca. 1,000 species), *Croton* (ca. 500–600 species), and *Phyllanthus* (ca. 400 species). Plant types range from herbs to trees and include vine- and cactus-like forms. Useful commercial products other than oils obtained from the family include rubber (*Hevea brasiliensis* Muell. Arg.), candelilla wax (*Euphorbia antisyphilitica* Zucc.), and cassava (*Manihot esculenta* Cranz.). Many species are grown domestically as ornamentals. The samples analyzed represent two of the four subfamilies and 10 of the 11 tribes within these subfamilies.

Materials and Methods

Collection, preparation, analysis of seed, and GLC analysis of the fatty acids were accomplished as previously described (5,15,18). Seed of *Euphorbia lagascae* was collected from wild plants in Spain under Public Law 480 funds. Methyl esters were prepared by acid-catalyzed methanolysis except for the *E. lagascae* preparation that was catalyzed by sodium methoxide (16).

[1] Presented at the AOCS in New Orleans, 1964.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, USDA.
[3] ARS, USDA.