Determination of Petroselinic Acid by Microreactor Chromatography

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ABSTRACT

The microreactor-ozonolysis technique was applied to the quantitative determination of the relative amounts of petroselinic and oleic acids in seven Umbelliferae seed oils. The operation was easy and rapid. Results were excellent when the method was tested on ester mixtures of known composition. When used on esters prepared from Umbelliferae seed oils, the method gave results comparable with those found by another procedure, also described, which combined thin-layer chromatography with either gas-liquid chromatography or ultraviolet spectrophotometry.

INTRODUCTION

In studying the composition of seed oils from many species of plants, investigation of the family Umbelliferae has been deferred because no rapid, reliable method was available for determining petroselinic acid in the presence of oleic acid. Older methods which utilized ester distillation and lead-salt separations (1) were recognized as containing certain errors (2). More recently oxidation with permanganate-periodate and analysis of the oxidation products by gas-liquid chromatography (GLC) (3) or by column chromatography (4) have proved useful. Ozonolysis, followed by GLC, appears to constitute a further improvement (5).

Ozonolysis in the microreactor apparatus (MRA), developed by Davison and Dutton (6) for determining the location of double bonds in fatty acids, has now been successfully applied to the quantitative determination of petroselinic acid in seed oils of the Umbelliferae. Results are compared with another method, developed in this laboratory, utilizing thin-layer chromatography (TLC) and either GLC or ultraviolet spectrophotometry.

METHODS

Oils were extracted from the seed or seed plus pericarp with petroleum ether (bp 30-60C) (7). The oil was saponified, and unsaponifiable matter was removed by the usual procedures (8). Free acids were recovered and esterified with diazomethane or with 5% HCl-methanol (w/v).

Microreactor

Ozonolysis of methyl esters and pyrolysis of the ozonides were accomplished in an MRA, and products were injected directly into the column for GLC as previously described (6). The 30x1/4-in. aluminum column was packed with 20% poly(ester-acetal) (9) or 30% diethylene glycol succinate on 40-60 mesh Chromsorb W.

The C_6 and C_9 aldehyde-ester ozonolysis products of methyl oleate and methyl petroselinate were used for quantitation since these products elute from the chromatographic column as single components whereas the C_{12} and C_9 aldehydes often are superimposed on other ozonolysis products and require correction factors.

The suitability of the procedure was demonstrated by analysis of mixtures containing known amounts of methyl petroselinate and methyl oleate (A, B, C, Table I), which were prepared from Hormel standard samples, and of two mixtures containing methyl palmitate and methyl linoleate in addition (D, E). The amount of C_9 aldehyde-ester derived from methyl linoleate was considered in calculating the amount of methyl oleate in samples D and E.

A similar correction for methyl linoleate was made for the Umbelliferae seed oil methyl esters. In a second analysis of the Umbelliferae esters and of standards D and E, the 18:1 fractions were isolated by preparative GLC in an aerograph A-700 "Autoprep," equipped with a 10 ftx1/4 in. copper column packed with 15% LAC-2-R 446 on 40-60 mesh Celite 545 run isothermally at 180C. A 12-μl injection yielded more than enough 18:1 (approximately 6 mg) for analysis with the MRA. The 18:2 fraction from *Daucus guttatus* esters was isolated in the same manner as the 18:1 fraction.

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TABLE I

Determination of Methyl Petroselinate in Standard Mixtures and in Mixed Esters from Seed Oils

Sample	Methyl Petroselinate in Total 18:1[a]			
	Wt %	TLC %	MRA %	MRA-Prep. GLC %
Standard A	87.8		84	
Standard B	50.7		52	
Standard C	10.6		11	
Standard D[b]	74.2		76	76
Standard E[c]	25.4		27	26
Actinolema macrolema Boiss.		62[d]	70	62
Bupleurum croceum Fenzl.		58[d]	63	57
Daucus guttatus Sibth. & Sm.		81[d]	86	84
Ducrosia anethifolia Boiss.		85[e]	84	84
Eryngium eburneum Decne.		93[d]	94	94
Oenanthe globulosa L.		99[e]	96	94
Petroselinum crispum (Mill.) Mansf.		93[e]	94	94

[a] Average of at least two analyses.

[b] Contains 9% 16:0 and 9% 18:2.

[c] Contains 13% 16:0 and 10% 18:2.

[d] TLC-UV method.

[e] TLC-GLC method.

Preparative TLC Methods

Used with GLC. TLC plates, 10x34 cm, were spread with 1-mm layers of diethyl-ether-washed Silica Gel G impregnated with 30% silver nitrate. Each plate was spotted with 25 (0.2 μl each) samples of Umbelliferae methyl esters. The chromatogram was developed with benzene in a 14x45-cm cylinder. Movement of the solvent front to 32 cm completely separated methyl petroselinate from methyl oleate. The bands containing the separated esters were scraped into individual flasks, and the adsorbent was extracted three times with ether. The solution was filtered, and the ether was removed under nitrogen. Ten milligrams of methyl myristate were weighed into a one-ml volumetric flask, and one of the recovered 18:1 fractions was quantitatively washed into this flask with ether. The flask was filled to the mark, and a 5-μl aliquot was analyzed by GLC in an F&M gas chromatograph, Model 810, equipped with a flame ionization detector and a 10 ftx1/8 in. copper column packed with 20% LAC-2-R 446. The other fraction of 18:1 was treated similarly, and the proportion of each was calculated by the following equations:

$$\frac{\text{Area 18:1 peak}}{\text{Area 14:0 peak}} \times \text{wt 14:0} = \text{wt 18:1}$$

$$\frac{\text{Wt } 18{:}1^{6} \times 100}{\text{Wt } 18{:}1^{6} + \text{wt } 18{:}1^{9}} = \%\ 18{:}1^{6} \text{ in } 18{:}1$$

Used with UV. TLC separation of methyl petroselinate and methyl oleate was carried out as for GLC except that the ether was distilled from sodium hydroxide pellets before extraction of the components. The esters were washed into individual one-ml volumetric flasks with spectral grade hexane and made to volume with this same solvent (no internal standard is used). These solutions were analyzed on a Beckman DK-2A recording spectrophotometer, which was continuously purged with dry nitrogen. The absorbance was determined at 182 mμ [molar absorptivity is 13,000 for both methyl petroselinate and oleate (10)], and the percentage of methyl petroselinate in the total 18:1 was calculated by the equation:

$$\frac{\text{Absorbance } 18{:}1^{6} \times 100}{\text{Absorbance } 18{:}1^{6} + \text{absorbance } 18{:}1^{9}} = \%\ \text{methyl petroselinate in total 18:1}$$

RESULTS AND DISCUSSION

The MRA procedure provided accurate analytical data on the five mixtures of known composition (Table I). Comparison of results from direct determination on Samples D and E, with data on the 18:1 fraction isolated by preparative GLC, indicates that the presence of methyl palmitate introduces no error, that the correction for methyl linoleate is entirely adequate, and that there is no fractionation of methyl oleate and methyl petroselinate in the preparative GLC.

In general, analyses of Umbelliferae esters either by direct MRA or by preparative GLC, followed by the MRA, gave results comparable with the TLC methods (Table I). Usually,

where results were not as close as desired, addition of preparative GLC helped to reconcile the percentages from the MRA and TLC methods. Since the esters were free of unsaponifiable material and since no unusual components were observed by GLC, the reason for the analytical differences is unknown. It was suspected that the 18:2 might include a component with Δ6 unsaturation which would make correction for methyl linoleate invalid; but isolation of the 18:2 component of *Daucus guttatus* by preparative GLC and location of its double bonds by MRA showed no Δ6 unsaturation present.

The MRA method is more straightforward and less time-consuming than the TLC method. The time required for the MRA method is about one-half hour whereas TLC plates require about three hours for the solvent to migrate 32 cm and another hour for the other steps in the method to be completed. Both methods are equally successful in determining the relative amounts of methyl petroselinate and methyl oleate.

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REFERENCES

1. Hilditch, T. P., and P. N. Williams, "The Chemical Constitution of Natural Fats," 4th ed., John Wiley and Sons Inc., New York, 1964, p. 287.
2. Grindley, D. N., J. Sci. Food Agr. *1*, 53 (1950).
3. Mallard, T. M., and B. M. Craig, JAOCS *43*, 1-2 (1966).
4. Chobanov, D., E. Chooparova, A. Popov and D. Kamenova, Fette, Seifen, Anstrichmittel *68*, 447–449 (1966).
5. Moreau, J. P., R. L. Holmes, T. L. Ward and J. H. Williams, JAOCS *43*, 352–354 (1966).
6. Davison, V. L., and H. J. Dutton, Anal. Chem. *38*, 1302–1305 (1966).
7. Earle, F. R., E. H. Melvin, L. H. Mason, C. H. Van Etten and I. A. Wolff, JAOCS *36*, 304–307 (1959).
8. AOCS Official Method Ca-6a-4.
9. Davison, V. L., and D. J. Moore, in preparation.
10. Binder, R. G., L. A. Goldblatt and T. H. Applewhite, J. Org. Chem. *30*, 2371–2376 (1965).

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