Chromatographic Analysis of Seed Oils. II. Fatty Acid Composition of Dimorphotheca Oil

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Abstract

The fatty acid composition of *Dimorphotheca sinuata* seed oil was determined by use of column and gas-liquid chromatography (GLC), and UV, IR, and nuclear magnetic resonance (n.m.r.) spectroscopy. The presence of a small amount of epoxy esters was confirmed by n.m.r. spectroscopy in conjunction with GLC and TLC. The presence of ca. 2.5% of 9-keto-*trans,trans*-10,12-octadecadienoic acid, previously unrecognized as a constituent of dimorphotheca oil, was established. The estimated fatty acid composition of dimorphotheca oil is 66.5% dimorphecolic, 14% linoleic, 10% oleic, 4.5% palmitic and stearic, and 5% miscellaneous acids.

Introduction

In the course of an extensive screening program in search of new and potentially useful industrial oils, the seed oils from several members of the family Compositae were shown to possess unusual properties. In particular, the seed of *Dimorphotheca sinuata*, previously referred to as *D. aurantiaca* (6,10,11,12, 15), yielded an oil ca. 48% of whose acids contained *trans, trans* conjugated diene (6). This dienoic acid was subsequently characterized as the optically active 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid and was named dimorphecolic acid (15). The unique α-hydroxydiene structure of dimorphecolic acid, and the relatively large proportion in the oil, suggested that dimorphotheca oil might have considerable industrial utility. Accordingly, preliminary developmental research on *Dimorphotheca* was undertaken. Several reports (6,10,11,12,15) have dealt with the proportion of various fatty acids in dimorphotheca oil. However, to aid the evaluation of the oil for potential utility, a comprehensive examination of its fatty acid composition was undertaken. This paper reports on the methods used and the results obtained.

Experimental

Materials and Methods. The seed used as a source of oil was procured by the New Crops Research Branch, ARS, USDA. The oil investigated was obtained from one lot of ca. 100 lb of seed of *D. sinuata* which, preliminary evaluation indicated, had a high proportion of conjugated diene. The following characteristics were noted for the oil used for analysis:

$d_4^{25} = 0.9537$, $n_D^{25} = 1.4916$, $\alpha_D^{25.5} = +6.6°$ (10 cm tube),
OH value = 116, a = 69.0 at 231 mμ

The oil was converted to acids or methyl esters which were separated into fractions by partition chromatography on silicic acid, and the proportion of the various fractions was determined by titrating acids and weighing esters (3). The proportion of nonhydroxy acids was determined chiefly by GLC. Additionally, UV, near IR, and n.m.r. spectra and TLC were used to determine the presence or relative proportion of hydroxy, keto, epoxy, and conjugated acids.

Preparation of Acids and Methyl Esters. Saponification was accomplished by allowing a mixture of 20.2 g dimorphotheca oil, 20 ml 95% ethanol, 20 ml water, and 6.5 g KOH pellets to stand 20 hr at room temp under nitrogen with 4 mg butylated hydroxytoluene added as inhibitor. Nonsaponifiables (0.27 g) were removed by extraction with ether. The soap solution was cooled to OC and acidified with ice-cold 3*N* HCl. The liberated acids were extracted with ether. Traces of HCl were removed by washing the ether extracts with water. After the ether solution was dried with sodium sulfate, solvent was removed under reduced pressure below 35C to give 18.95 g fatty acids. In contradistinction to the results of Smith et al. (15), there was no apparent loss of conjugated diene, nor did conjugated triene increase during preparation of the acids, as judged by the intensity of characteristic absorption in the UV.

Methyl esters were prepared in two ways: esterification of the free fatty acids with diazomethane, and base-catalyzed transesterification of the glycerides with methanol in an Amberlite 401 ion-exchange resin column.

Chromatography, Titration and UV Analysis of Acids. Dimorphotheca acids were chromatographed on a silicic acid column and 10-ml portions of eluate were collected and titrated as described previously for castor acids (3). A sample of 0.52 g was resolved into three fractions which constituted 31.7,67.5 and 0.8 mole % (Fig. 1). Another sample of 0.84 g was resolved similarly into three fractions of 32.1,67.3 and 0.6 mole %. Recoveries were 99.0% and 98.5%, calculated on the basis of titration of aliquots of the solutions placed on the columns. The first fraction consists of unsubstituted fatty acids, the second of oxidized fatty acids (hydroxy, keto, and epoxy), and the third, which was not investigated closely, seems to contain polymeric and more highly oxidized acids. Selected portions were examined for characteristic UV absorption. After the 10-ml portions were titrated, the solvent was evaporated, methanol was added to the residues, and UV spectra of the methanol solutions were taken. Portions in the first fraction showed characteristic maxima at 259, 268 and 279 mμ, indicating the presence of conjugated triene. Similar examination of portions of the second fraction indicated a major amount of a conjugated diene with absorption at 231 mμ and a minor amount, concentrated in the earlier 10 ml portions, of a component which exhibited a single maximum at 275 mμ and was subsequently shown to be 9-keto-*trans,trans*-10,12-octadecadienoic acid.

Calculations based on the acid content of the various 10-ml portions from the second fraction, as determined by titration, the observed intensity of the absorption of each portion at 231 mμ and 275 mμ, and the molar absorptivities of pure dimorphecolic acid (λ 231 mμ, $\epsilon = 33600$) and of pure 9-keto-10,12-octadecadienoic acid (λ 275 mμ, $\epsilon = 29000$) indicated the presence of 64.7 mole % and 2.3 mole %, respectively, for these two acids in the mixed fatty acids of dimorphotheca oil. Calculation also indicated that the sum of the dimorphecolic and keto-dienoic acid was about 0.4% less than the total amount (titer) of acid in this fraction. The UV spectrum of the third

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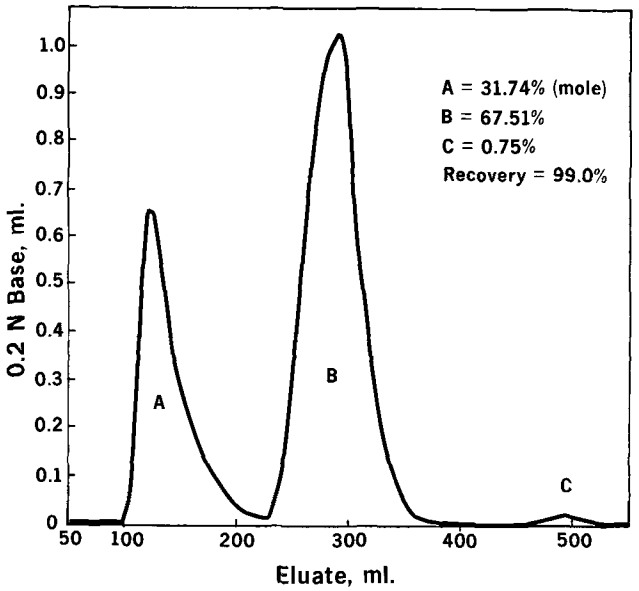

FIG. 1. Titrimetric analysis of unsubstituted acids (A), epoxy, keto and monohydroxy acids (B), and polyhydroxy and polymeric acids (C) obtained by fractionation of the fatty acids of dimorphotheca oil.

fraction indicated the presence of a small amount of conjugated diene and triene. In order to help characterize this fraction, another sample of dimorphotheca acids was chromatographed. The material in this fraction constituted 1.3% of the sample by weight and had a neutralization equivalent of about 550. The material recovered from the titrated solution was developed on chromatostrips (1). This revealed the presence of several components, the predominant ones being more polar than dihydroxystearic acid.

Characterization of the Keto-diene. The material used for characterizing the keto-diene was isolated from mixed dimorphotheca methyl esters by low-temp crystallization. This ester, after purification by silicic acid chromatography and crystallization from commercial pentane, melted at 33.0–34.0C. Anal.: calcd. for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 74.2; H, 10.3.

The UV spectrum of this ester, Figure 2, showed a single sharp maximum at 275 mμ in methanol (a = 94.2 l/g-cm) and at 267 mμ in cyclohexane (a = 94.2 l/g-cm). This displacement of position of maximum absorption of conjugated ketones is a recognized characteristic and, according to Fieser (7), amounts to about 11 mμ when methanol is replaced by hexane as the solvent. The IR spectrum of a solution in CCl_4 is shown in Figure 3. Especially noteworthy are the bands at 5.93,6.00,6.11,6.28 and 10.05 μ associated with the keto-diene system, in addition to the 5.75 μ band resulting from ester carbonyl stretching.

Hydrogenation in acetic acid with platinum oxide catalyst yielded methyl 9-hydroxystearate which, after crystallization from pentane, melted at 50.0–50.2C, lit mp 50.3–50.6C (2). This product was oxidized with chromic anhydride in acetic acid (4,14,15) to give methyl 9-ketostearate which, after crystallization from pentane, melted at 47.4–48.0C, lit mp 47.5–48.0C (2).

The keto-dienoate was synthesized by oxidizing methyl dimorphecolate with chromic anhydride in pyridine (13,15) and purified by crystallization from pentane. IR and UV spectra of the synthetic sample were identical to those of the natural material, and the mp of a mixture was not depressed.

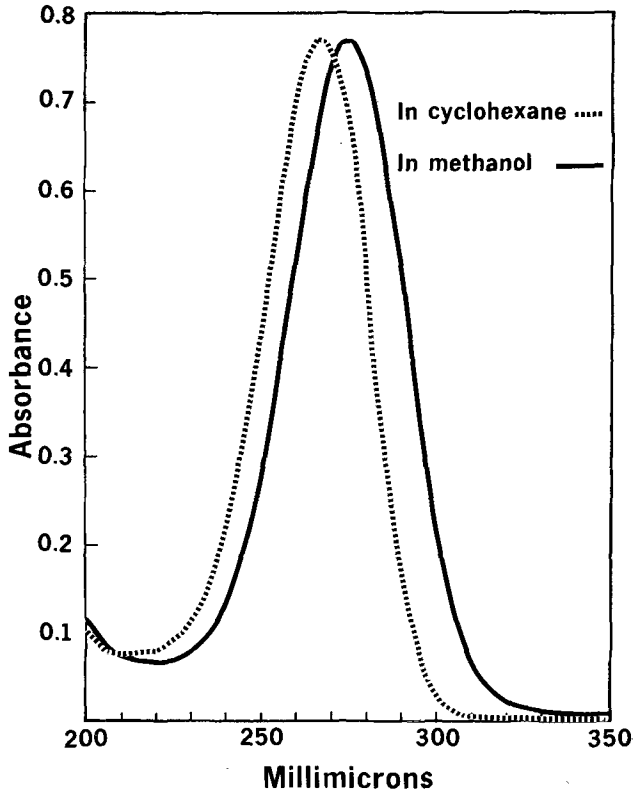

FIG. 2. Solvent effect on UV spectra of methyl 9-keto-10,12-octadecadienoate (8μl/10 ml, 0.1-cm cell).

Analysis of Chromatographed Esters. The conditions used for partition chromatography of methyl esters were somewhat different from those used for chromatography of the acids. Typically, the column was prepared with only 35 ml 20% methanol in benzene for 50 g silicic acid, the mixture was slurried with 100 ml benzene, and the esters were eluted with 350 ml 1% methanol in benzene, followed by 150 ml 4% methanol in benzene.

Good separation of unsubstituted esters, epoxy esters, and hydroxy esters was attained and appropriate portions, as determined by chromatostrip monitoring (1), were combined into three fractions. Fraction I (ca. 120 ml eluate) consisted of unsubstituted esters. For esters prepared from dimorphotheca acids with diazomethane, this fraction made up 30.8% of the sample weight. The UV spectrum of a methanol solution of this fraction shows characteristic absorption for conjugated triene (ca. 270 mμ) but no maximum at 231 mμ indicative of conjugated diene. Although the conjugated triene component was not isolated and characterized, the position of the maxima

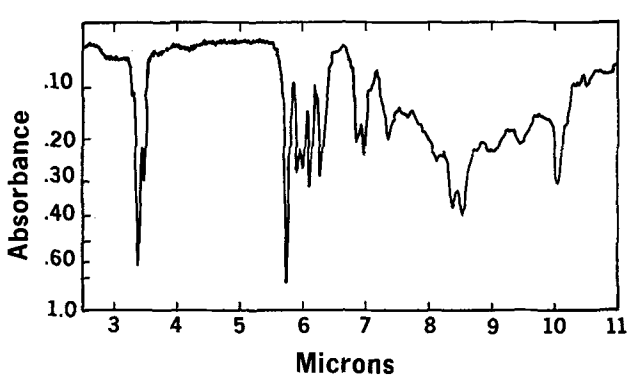

FIG. 3. IR spectrum of methyl 9-keto-10,12-octadecadienoate (CCl_4 solvent, 48.1 mg/10 ml, 1-mm cell, Model 137 infracord, NaCl optics).

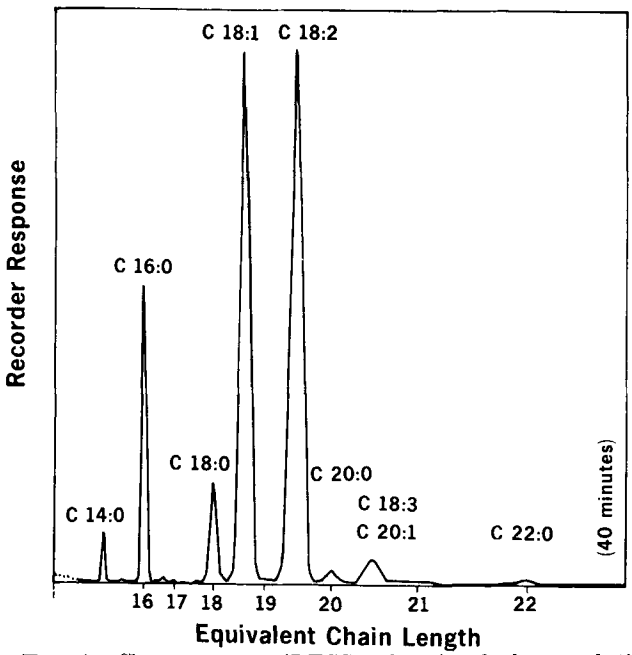

FIG. 4. Chromatogram (DEGS column) of the unsubstituted esters from dimorphotheca oil.

at 259,268, and 279 mμ, in methanol, suggests the all *trans* configuration for the triene (8,9). The IR spectrum shows a distinct maximum at 10.34 mμ indicative of the possible presence of some *cis* isomer (8). The intensity of the peak at 268 mμ indicates the presence of 4.0% conjugated triene, calculated as methyl β-eleostearate, in this fraction.

After chromatographing the esters obtained by transesterification, the fraction which contained the unsubstituted esters was analyzed by GLC. An Aerograph, model A-90-A.C., equipped with a 1-mv Leeds and Northrup recorder and a disc integrator and fitted with either a polar or nonpolar column, was used to analyze the esters. The polar column was a 6-ft, 0.25-in., stainless steel column packed with 15% diethylene glycol succinate (DEGS) on 60–80 mesh Gas Chrom P. This was used at 200C with a helium flow rate of 60 ml/min. The nonpolar column was a 5-ft, 0.25-in. stainless steel column packed with 20% Apiezon L on 60–80 mesh Gas Chrom P. This was used at 250C with a helium flow rate of 60 ml/min. Esters were identified and estimates of the proportion present were made as previously described (3). A chromatogram of the unsubstituted esters, through those with equivalent chain length of 22.5, is shown in Figure 4. Another portion of the unsubstituted esters was hydrogenated (in acetic acid with platinum oxide catalyst), and the hydrogenated esters were also analyzed by GLC. The estimated composition before and after hydrogenation is given in Table I.

The next 50 ml of eluate, following Fraction I, obtained on partitioning the esters prepared by means of diazomethane were combined to form Fraction II. Analysis of these more polar esters (ca. 3% of the sample weight), which included some of the keto-diene but no dimorphecolate, is discussed in the next section. The remaining eluate (ca. 300 ml) was combined to form Fraction III, which contained mostly methyl dimorphecolate and did not include the highly polar material corresponding to fraction 3 in the chromotography of the acids. Analysis of this fraction by GLC and TLC did not disclose another hydroxy ester.

Epoxy Acids. The presence of epoxides in dimorphotheca oil has been reported (6,10,11,12). In fact, the paper announcing the discovery in the oil of an acid with both an hydroxyl group and conjugated diene reported the presence of 52.0% oxirane oxygen as epoxy oleic acid (6). This was based on absorption of hydrogen bromide which, as the authors suspected, gave a false indication of oxirane oxygen. Later, Morris and Holman (12), after measurement of near IR absorption caused by chlorohydrins produced from epoxides, estimated that 0.6% of epoxy acids is present in oil from *D. aurantiaca*. Morris et al. (11) concluded, on the basis of GLC and TLC analyses, that five different epoxy acids are present, proposed structures for four of the acids and concluded that the fifth, which comprised about 0.3% of the acids of dimorphotheca oil, contains a *trans, trans* conjugated diene structure.

TABLE I

Estimated Composition of Unsubstituted Esters (Fraction I)

Compound	Before hydrogenation %	After hydrogenation %
C14:0	1.0	0.9
C15:0	0.1	0.1
C16:0	9.0	9.2
C16:1	0.2	
C17:0	0.1	0.3
C17:1	0.1	
C18:0	5.3	87.1
C18:1	31.4	
C18:2	45.0	Σ = 87.3
C18:3	1.6[a]	
C18:3 conjugated	4.0[b]	
C19:0	0	0.2
C20:0	0.9	1.8
C20:1	0.9[c]	
C22:0	0.4	0.4

[a] Obtained as difference between amount of component with equivalent chain length (ELC) 20.5, as in Fig. 2, and increase in C20:0 following hydrogenation.
[b] Obtained by UV analysis.
[c] Calculated as increase in C20:0 following hydrogenation.

In investigating the possible presence of epoxy acids, methyl esters prepared both by transesterification and by esterification of the mixed acids with diazomethane were examined. The esters prepared with diazomethane were free of nonsaponifiables, but they might have included artifacts formed from diazomethane, e.g., polymethylenes and the ether formed by reaction of hydroxyl with diazomethane (3). The esters prepared by transesterification may be expected to contain nonsaponifiables.

The expected ether (methyl 9-methoxy-10,12-octadecadienoate) was detected in the esters produced by means of diazomethane and also by transesterification. This was established by observation of characteristic absorption at 231 mμ in selected portions of eluate from partition chromatography and also by GLC, TLC, and IR analysis. The Fraction II obtained from the esters produced by transesterification (A) comprises 3.0% of the sample weight taken, and the corresponding fraction obtained from the esters prepared with diazomethane (B) amounted to 3.2%. Nuclear magnetic resonance spectra of a 10% solution of A in CCl_4 established the presence of epoxy protons in this sample showing the characteristic quintuplet in the interval of 7.12–7.42 tau with its center at 7.27. The presence of linoleate, with characteristic triplet at 7.19–7.36 tau and center also at 7.27, makes recognition and estimation of epoxy content most difficult. In fact, the characteristic epoxide quintuplet is completely masked if the whole mixed esters or dimorphotheca oil is used, but it was estimated (on the basis of NMR and GLC data) that a maximum of 14% of epoxy esters (as epoxy oleate) is present in A. This corresponds to about 0.4% as the maximum epoxy ester content of the dimorphotheca oil.

TABLE II
Equivalent Chain Length (ECL) and R_f Values for Methyl Esters Used as Reference Compounds

Compound	ECL on DEGS	ECL on Apiezon	R_f[a]
Oleate	18.6	17.7	
Linoleate	19.4	17.6	.74
Linolenate	20.5	17.6	
12-Methoxystearate	20.8	19.0	.60
12-Methoxyoleate	21.4	18.7	.62
α-Eleostearate	23.4	19.6	.70
9-Methoxy-10,12-octadecadienoate	23.4	decomposed	.56
cis-9,10-Epoxystearate	23.5	19.4	.52
Vernolate	24.2	19.1	.55
9-Ketostearate	24.5	19.3	.55
9-Hydroxystearate	25.3	19.5	.16
Dimorphecolate	28.8	decomposed	.16
9-Keto-10,12-octadecadienoate	29.0	20.9	.41

[a] The R_f's were determined by the use of 12 x 140 mm chromatostrips coated with a 300-μ layer of silica gel G. After development with 20% ether in pentane in a stoppered 18 x 150-mm test tube, the chromatostrip was sprayed with dilute H_2SO_4 and charred.

The NMR spectra also indicated the presence of methoxy (ether) protons in A. This is doubtless due to the presence of the 9-methoxy compound mentioned previously. UV absorption analysis indicated the presence of about 19% conjugated diene in A and 21% in B. The IR spectrum of A shows absorption at 9.11 μ, characteristic of the C-O stretching of ethers. When fractions A and B were chromatographed on a 4-ft DEGS column at 175C, the peak for the ether was identified by UV analysis of column effluents. Strong UV absorption at 231 mμ was observed for the component with an equivalent chain length (ECL) of 23.4. This ECL and the R_f on chromatostrips of this component of Fraction II are the same as for the ether synthesized from methyl dimorphecolate (Table II).

The composition of fractions A and B was further investigated by GLC (both DEGS and Apiezon L columns) and TLC (chromatostrips and chromatoplates) coupled with examination of UV spectra when this seemed appropriate. These experiments revealed the presence of a considerable amount of the keto-diene, methyl 9-keto-10,12-octadecadienoate, as well as of the ether and smaller amounts of unsubstituted fatty esters and epoxy esters. The epoxide present in larger amount had an ECL of 24.0 on DEGS and 19.0 on Apiezon L, R_f of 0.55 (chromatostrip), and no characteristic absorption in the range 220–300 mμ. These characteristics suggest a C_{18} epoxy ester with one double bond. Another component which had an ECL of 24.8 on DEGS and 19.2 on Apiezon L, an R_f of 0.50, and showed no characteristic UV absorption above 220 mμ may well be a C_{18} epoxy ester with two double bonds. Both A and B contained a small amount (ca. 0.1% of the whole dimorphotheca esters) of a component with an ECL of 27.2 on DEGS and 19.8 on Apiezon L, an R_f of 0.40, and strong characteristic absorption at 279.0 mμ in methanol. These characteristics suggest a conjugated keto-diene structure. But this compound and the epoxides, comprising altogether less than 0.5% of the methyl esters of dimorphotheca oil, were not characterized further.

Table II shows values for the R_f on chromatostrips and the ECL on DEGS and Apiezon L of a number of reference compounds used as an aid in identifying various components.

Table III gives our best estimate, based on results obtained in all operations described here, for the fatty acid composition of dimorphotheca oil.

Discussion and Conclusions

The fatty acid composition was calculated from results of UV, silicic acid chromatographic, and GLC analyses. Mole % values obtained by titration were converted to wt % after determining the average mol wt of the relevant fractions. The average mol wt of the least polar fraction of the acids was calculated from the weight percentage of each component as determined by GLC of Fraction I of the esters. For the second, more polar, fraction of the acids, the average mol wt was based on the UV data. The values in Table III are exclusive of the small amount (ca. 0.7 mole %) of unidentified highly polar components.

TABLE III
Estimated Fatty Acid Composition of Dimorphotheca Oil

Acid	% by weight
Dimorphecolic	66.4
Linoleic	13.8
Oleic	9.7
Palmitic	2.8
9-keto-10,12-octadecadienoic	2.3
Stearic	1.6
Trienoic, conjugated	1.2
Linolenic	0.5
Myristic	0.3
Arachidic	0.3
Eicosenoic	0.3
Epoxy, one double bond	0.3
Epoxy, two double bonds	0.1
Behenic	0.1
Keto-unsaturated (?)	0.1
Summation of C15:0, C16:1, C17:0, C17:1, and C19	0.2

Analyses for hydroxyl by determination of the intensity of hydroxyl absorption in the near IR (2.76 μ) and for conjugated diene content of various portions obtained by partition chromatography indicated that all (to within 1%) of the acids containing conjugated diene with characteristic absorption near 230 mμ also contain an hydroxyl group and conversely, all the acids that contain an hydroxyl group also contain conjugated diene in the chain. Although the seed oil of another member of the family Compositae, *Tragopogon porrifolius L.*, was found to contain both 9-hydroxy-10, 12- and 13-hydroxy-9, 11-octadecadienoic acids (5), the latter was not found in dimorphotheca oil by Morris et al. (10) nor by us.

That the keto-diene is in fact a component of dimorphotheca oil and is not an artifact was established by noting the difference in position of maximum absorption in the region of 270 mμ when the oil is dissolved in cyclohexane and in methanol, resulting from the different position of maximum absorption, 268 and 275 mμ, of the keto-diene moiety in the two solvents.

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