in "triolein." Olive oil is generally considered to contain about one-third of the former and two-thirds of the latter.

ACKNOWLEDGMENT

IR measurements for *trans* isomers by G. J. Boudreaux and Sylvia H. Miles.

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Gas-Liquid Chromatography of Polar Fatty Derivatives[1, 2]

R. A. MORRISSETTE and W. E. LINK, Research Center, Archer-Daniels-Midland Company, Minneapolis, Minnesota

Abstract

The direct separation of fatty amides is achieved using a polyamide, Versamid 900, as the partitioning agent on a support which need not be previously impregnated with strong alkali or acid, procedures usually followed in the GLC separation of highly polar materials. The combination of a neutral support and polar substrate permits the separation of unsubstituted and substituted long chain fatty amides with as many as 24 carbon atoms with good resolution, in a reasonable time, and with good peak symmetry. The observed area responses agree with the wt percentages of standard amide mixtures, indicating that no loss of amides occurs on the column under the conditions employed.

The Versamid column has proved useful in the analysis of other polar fatty derivatives. Conjugated dienoic and trienoic acids run as their methyl esters are retarded sufficiently on Versamid 900 so that they may be estimated in the presence of other fatty acids. Mixtures of compounds with varying polarity, such as mono-, di- and triacetin, and glycerol, may be separated easily. Hydroxy and normal fatty acid methyl esters give equally symmetrical peaks.

Introduction

THE DIRECT SEPARATION of long chain fatty amides by gas chromatography has received little attention. The usual practice has been to hydrolyze the amides to acids which may be readily separated using conventional techniques. Metcalfe (5) showed that phosphoric acid treated polyester columns could be used to analyze amides, but observed that nitrile formation took place on the column. Subsequently these workers (6) obtained good separations of fatty amides with alkaline treated columns that were previously employed for the analysis of fatty amines (4), but no quantitative data were provided.

The object of our work was to produce a column for amide separations which would require neither acid nor alkali pretreatment, avoiding loss of the amides because of reaction with the column substrate. The rule of similarity suggested that a polyamide would serve as a stationary phase, and subsequent work with Versamid 900 resulted in a column which would resolve fatty amides and allow a response for those impurities associated with amides, such as nitriles and esters. This substrate also proved useful for mixtures with components having a wide range of polarity and for conjugated acids, such as those found in dehydrated castor oil.

[1] Presented at the AOCS meeting, Minneapolis, 1963.
[2] Technical Paper, No. 259, ADM Co.

Experimental

Column Preparation. Two combinations of partitioning agent, Versamid 900, and solid support were used, either 20% w/w on 60–80 mesh or 5% w/w on 100–120 mesh Gas Chrom P. The particular combination used in an experiment is described in the accompanying figures. *n*-Butanol-chloroform (1:1) was found to be an effective solvent for the polyamide. The column material was packed into 4–6 ft aluminum tubing 0.25 in. OD. To obtain max temp stability for temp programming, the column was heated at 350C with a flow of nitrogen for 12 hr. The conditioned column was ready for operation min after insertion into the gas chromatograph.

Instrumental Conditions. An F & M Model 500 gas chromatograph was used in the work, with the bridge operated at 200 ma, detector block maintained at 300C, and a flow rate as described later. The analyses may be conducted isothermally, but the use of temp programming facilitates the identification of low-boiling impurities and increases the resolution of the amides. Since the amides are extremely high boilers it is important to maintain a high temp in the inlet, 275–400C. Care should be taken to insure that the inlet is not contaminated with impurities which would cause dehydration of the amides to cor-

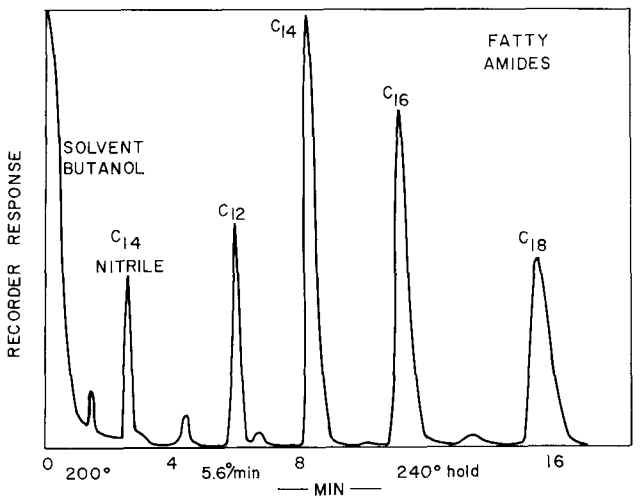

FIG. 1. Chromatogram of fatty amide from high cut coconut fraction, 5 ft column, 20% Versamid 900 on 60–80 mesh Gas Chrom P, temp programmed from 200C at 5.6°/min to hold at 240C, inlet 300C, detector 300C, flow rate 100 ml/min.

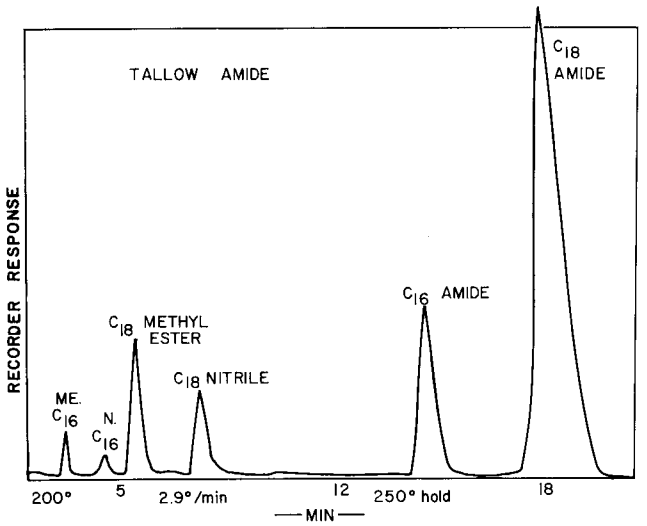

FIG. 2. Chromatogram of tallow amide, 5 ft column, 20% Versamid 900 on 60–80 mesh Gas Chrom P, temp programmed from 200C at 2.9°/min to hold at 250C, inlet 300C, detector 300C, flow rate 100 ml/min.

responding nitriles. A solid sampler can be used to inject 1–2 ml of the high melting amides, or 5–10 μl of a 10% solution of the amide in butanol can be injected with a liquid sampler.

Results and Discussion

Amides. A chromatogram of a high cut coconut amide fraction is shown in Figure 1. The column was temp programmed from 200–240C at a rate of 5.6C/min, resulting in a retention time of 15 min for stearamide. Nitrile contaminants are readily discernible. A tallow amide is shown in Figure 2, with nitriles and methyl esters present as extraneous material. The extreme conditions necessary for the analysis of a commercial fatty amide containing a high percentage of erucyl amide are illustrated in Figure 3.

The amount of nitrile displayed in the chromatograms has been found to depend on two factors: the condition of the inlet and the level of nitrile originally present as the accompanying impurity. It was observed that the dehydration occurring during the separation was independent of the column temp but increased as the inlet became fouled. Replacement of the metal inlet system with an inert material, such

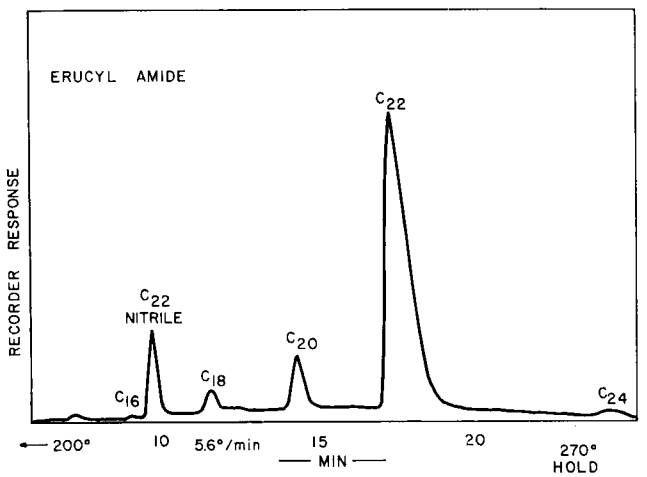

FIG. 3. Chromatogram of rapeseed amide, 4 ft column 5% Versamid 900 on 100–120 mesh Gas Chrom P, temperature programmed from 200C at 5.6°/min to hold at 270C, inlet 350C, detector 300C, flow rate 100 ml/min.

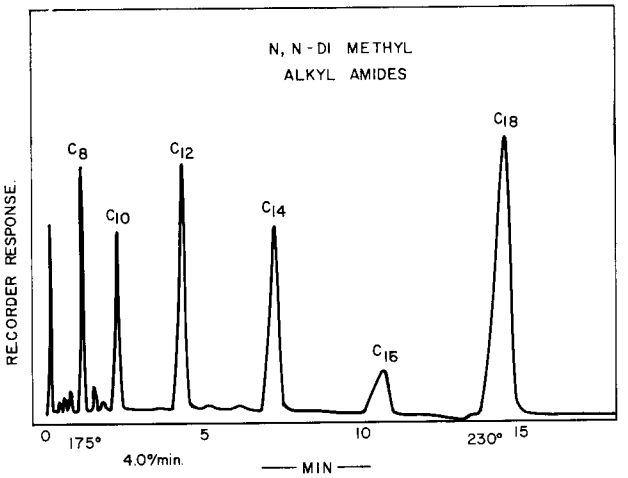

FIG. 4. Chromatogram of N,N-dimethyl akyl amides, 4 ft column, 5% Versamid 900 on 60–80 mesh Gas Chrom P, temp programmed from 175C at 4°/min, inlet 300C, detector 300C, flow rate 120 ml/min.

as quartz, should serve to eliminate much of this decomposition. The loss of each chain length amide through dehydration is proportional to the amt present so that the distribution of the amide as reflected by the area percentages, neglecting the nitrile peaks, is not affected. This was substantiated by hydrolyzing amide samples to the corresponding acids and determining the composition of the methyl esters on polyester columns. All components of the same chain length, saturated and unsaturated, were combined. The results from such a study of the materials referred to in Figures 1–3 show in Table I. The low values for the low-boiling components of the coconut amide are believed to be caused by a loss through volatilization during the hydrolysis of the amide in an acid medium.

With temp programming, the area percentages of the homologous amides were found to be directly related to the calculated compositions. No correction factors for detector responses were needed for the components encountered in natural products. Table II shows data obtained with known mixtures of pure amides. Precision values have not been calculated

TABLE I

Comparison of Compositions of Amides by Direct and Indirect Analysis

Mixture	Component	% Area as amides	% Area as methyl esters
Coconut	C_{10}	3.8	2.6
	C_{12}	62.3	57.1
	C_{14}	26.3	30.2
	C_{16}	6.1	8.1
	C_{18}	1.5	2.0
Tallow	C_{14}	3.2	3.3
	C_{16}	28.6	27.9
	C_{18}	67.1	67.6
	C_{20}	1.1	1.2
Rapeseed	C_{16}	Trace	0.3
	C_{18}	2.2	2.4
	C_{20}	8.4	8.0
	C_{22}	88.0	88.3
	C_{24}	1.3	1.0

TABLE II

Analysis of Known Mixtures of Amides

Mixture	Component	% By wt	% By area
1	C_{14}	50.5	51.8
	C_{12}	49.5	48.2
2	C_{14}	50.1	49.6
	C_{18}	49.9	50.4
3	C_{14}	52.2	53.1
	C_{16}	47.8	46.9
4	C_{14}	40.7	41.2
	Lauryl Lauramide	59.3	58.8
5	C_{12}	10.9	10.0
	C_{14}	53.0	53.7
	C_{16}	25.9	25.4
	C_{18}	10.2	10.8

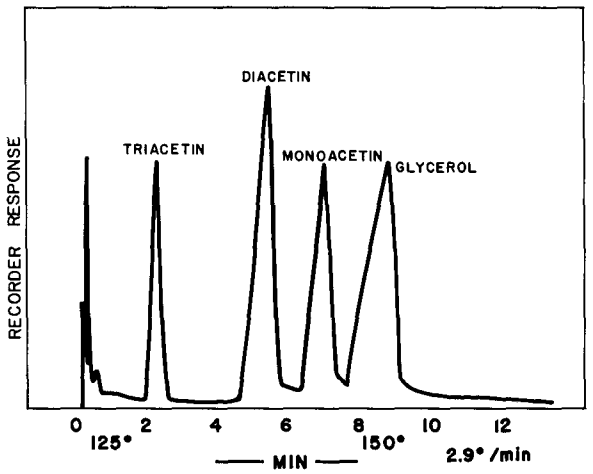

FIG. 5. Chromatogram of mixture of glycerol, monoacetin, diacetin, and triacetin, 5 ft column, 20% Versamid 900 on 60–80 mesh Gas Chrom P, temp programmed from 125C at 2.9°/min, inlet 300C, detector 300C, flow rate 100 ml/min.

but are believed to be comparable to those obtained in the analysis of fatty acids. The retention time of lauryl lauramide approximates that of C_{24} amide. The symmetry of the lauryl lauramide peak was good and no base-line drift was noticed.

N,N-dimethyl alkyl amides may be analyzed easily with the polyamide column. Figure 4 shows a chromatogram of a mixture of commercially available substituted amides, in which the alkyl group ranges from C_8–C_{18}.

Hydroxy and Conjugated Acids. Polyamide columns have been reported to be useful for the analysis of fatty alcohols and polyols. This has been confirmed and, in addition, satisfactory separations have been obtained with Versamid 900 of components with widely differing polarities. A reaction mixture of glycerol with acetic acid provides an example (Fig. 5) of this application. Polyacetates and esters up to a mol wt of 600 may be run simply by taking advantage of the stability of the polyamide and operating at higher temp. The polyamide column has been subjected to temp of 350C for several hr with no apparent damage.

Hydroxy acids, in products such as castor oil, constitute a special case. With polyester partitioning equal widths at the base line were attained. The sample was dissolved in methyl hexanoate. No attempt was made to perform a quantitative analysis on the mixture.

amide column a mixture, including methyl 12-hydroxy stearate and methyl 9,10-dihydroxy stearate, is resolved in 13 min, as shown in Figure 6. Using temp programming, peaks with good symmetry and almost agents, a high temp, a short column, or both are required to obtain symmetrical peaks and to prevent loss of the hydroxy component which occurs through interaction with the column material. With a poly-

TABLE III

Analysis of Conjugated Dienes—DCO by GLC

Sample No.	% Conj. diene (c,t/t,c;c,c;t,t) Polyester	Versamid	UV	% C_{18}OH Versamid
1	40.28	39.53	27.8	4.73
2	39.90	39.06	26.1	6.71
3	36.81	37.69	25.8	9.60
4	35.72	36.75	24.9	11.91
5	33.10	33.02	22.3	19.41
6	40.43	40.11	28.3	2.49
7	40.81	40.33	28.2	1.62
8	38.95	38.86	26.2	5.10
9	38.94	39.31	27.4	3.05

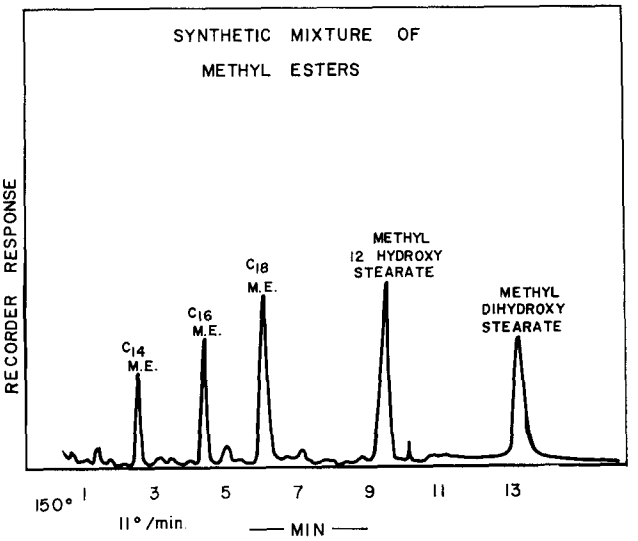

FIG. 6. Chromatogram of mixed methyl esters, 4 ft column, 5% Versamid 900 on 100–120 mesh Gas Chrom P, temp programmed from 150C at 11°/min, inlet 400C, detector 325C, flow rate 120 ml/min.

Conjugated fatty acids have longer retention times on Apiezon and polyester columns than the corresponding non-conjugated acids (1,3,8). Thus it is possible to analyze both types of acids simultaneously, except that with polyester columns non-conjugated trienes interfere with the conjugated dienes. Other functional groups, such as keto or hydroxy groups, make the separations extremely difficult. Figure 7 shows a dehydrated castor oil methyl ester run on a polyamide column. The conjugated peaks were identified through reference to a chromatogram of the same sample on a polyester column and to the data of Body and Shorland (2). The non-conjugated C_{18} acids are found with the saturated C_{18} acid and the conjugated dienes are eluted between C_{18} and C_{20} saturated acids. Methyl ricinoleate is eluted in 20 min during the isothermal separation.

Several dehydrated castor oils were analyzed for conjugated dienoic acids on polyester and on polyamide columns and the results were compared with those obtained by the UV method prescribed in AOCS Ka 13-56. Data appear in Table III. The polyamide

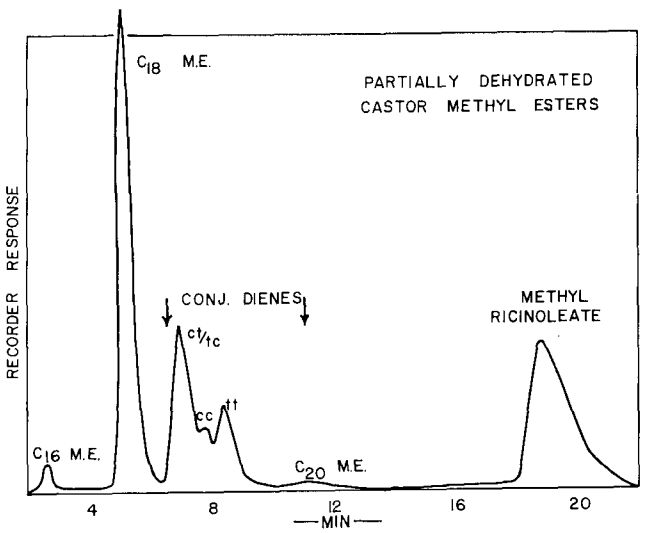

FIG. 7. Chromatogram of dehydrated castor oil methyl esters, 5 ft column, 20% Versamid 900 on 60–80 mesh Gas Chrom P, isothermal at 200C, inlet 300C, detector 275C, flow rate 100 ml/min.

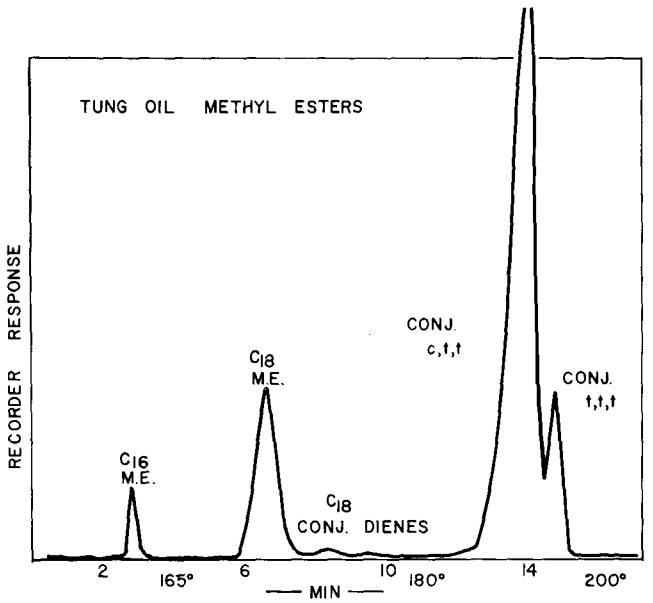

FIG. 8. Chromatogram of tung oil methyl esters, 4 ft column, 5% Versamid 900 on 100–120 mesh Gas Chrom P, isothermal at 165C for 5 min, temp programmed at 2.9°/min to 200C, inlet 300C, detector 275C, flow rate 100 ml/min.

column allows the unreacted hydroxy acid to be determined simultaneously while the polyester column does not. In all cases the GLC values are higher than the UV data and reflect more closely the predicted composition of the dehydrated oils. The reasons for the discrepancy are not yet known, but it is suspected that the absorption coefficient used for the UV analyses is not correct. The coefficient is a composite of the absorption coefficients of the conjugated dienes found in dehydrated castor oil.

The separation of α-eleostearic and β-eleostearic acid has been reported for both Apiezon (1,7) and polyester (7,8) columns with the α-eleostearic (c,t,t) eluting first. The order of elution is the same on a polyamide column, as shown in Figure 8. With temp programming the conjugated trienes are eluted in less than 15 min. The conjugated dienes precede the conjugated trienes, and the unconjugated components travel with the saturated components.

The retardation of the conjugated trienes, with respect to the unconjugated trienes, is such that a direct measurement of the total triene conjugation may be made, without recourse to UV spectrophotometric techniques which are at best only comparative. The situation is quite comparable to that encountered in the analysis of dehydrated castor oil.

The polyamide substrate has proved to be useful for the analysis of polar derivatives not readily separable on other columns by GLC. The versatility and stability of the polyamide when used as a column substrate at high temp will undoubtedly lead to other applications in the analysis of fatty derivatives.

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Composition of Insect Waxes. I. Waxes of Exotic Coccidae: *Gascardia madagascariensis, Coccus ceriferus* and *Tachardia lacca*

E. FAUROT-BOUCHET and G. MICHEL, Laboratoirede Chimie Biologique, Faculte Catholique des Sciences, Lyon, France

Abstract

Composition of several coccid waxes has been determined by means of alumina and gas-liquid chromatography. *Coccus ceriferus* wax is a mixture of the esters of C_{26} and C_{28} alcohols with C_{24},C_{26} and C_{28} acids. *Tachardia lacca* wax has a high percentage of free alcohols (essentially C_{28} alcohol); *Gascardia madagascariensis* wax contains a large proportion of free acids. In addition to C_{26},C_{32} and C_{34} normal chain acids, there are several C_{30},C_{32} and C_{34} hydroxy acids, in which the hydroxyl function is situated in the middle of the hydrocarbon chain. Small proportions of odd and even hydrocarbons are present in all of the waxes investigated.

Introduction

NATURAL WAXES have been the subject of considerable study, and the results of these investigations were reviewed some years ago by Warth (1). The methods previously available, such as crystallization and distillation, were inadequate for the fine separation of distinct homologous higher fatty acids, fatty alcohols, or hydrocarbons. For this reason, it was desirable to apply the new and precise chromatographic techniques now available for more accurate determination of the nature and proportions of these compounds. Gas-liquid chromatography (GLC) is particularly useful for this purpose, although its application to compounds with boiling points in the higher ranges present some experimental difficulties. In recent years, these methods have been applied by several workers to plant waxes (2,3,4); mineral waxes (5,6); and human waxes (9). It was our purpose to investigate the composition of some insect waxes, more precisely those of the Coccidae, or scale insects.

Materials and Methods

Commercial samples of China wax from the insect *Coccus ceriferus,* and of shellac wax from the Indian lac insect, *Tachardia* (or *Carteria,* or *Laccifer*) *lacca,* were available from several firms. Samples of waxy material from *Gascardia madagascariensis,* a coccid of Madagascar, were extracted from the cocoons of this insect.

Extraction of Waxes. Cocoons (1 kg) were pounded,