CHROM. 4830

HEPTADECENOIC ACID AS AN INTERNAL STANDARD IN THE GAS CHROMATOGRAPHIC WEIGHT DETERMINATION OF FATTY ACIDS

A. V. ZHUKOV AND A. G. VERESHCHAGIN
Lipid Biochemistry Research Unit, Institute of Plant Physiology, Academy of Sciences, Moscow (U.S.S.R.)

(Received May 6th, 1970)

SUMMARY

The method of calibration for the fatty acid methyl esters weight microdetermination (10–100 μg) by gas–liquid chromatography with an argon detector and dimethyldichlorosilane treated polyethyleneglycol adipate column is described. Methyl *n-cis*-9-heptadecenoate was used as an internal standard in the calibration. The acid was isolated from *Candida* sp. fat by liquid–liquid partition of the methyl esters, urea crystallization and preparative gas–liquid chromatography, and was identified by its melting point, refraction index, gas–liquid chromatography retention volume, oxidative degradation and infrared spectrum. Calibration was performed at different sample (soybean fatty acid methyl esters)/standard weight ratios. Good weight determination accuracy can be achieved in the 10.4–25.9 ratio range after multiplying the standard peak area by a correction coefficient. The corrected recovery value in the above range was 92.4–105.5%, with a relative standard deviation of $\pm$ 3.7%. Saponification of the sample–standard mixture, extraction of unsaponifiable and conversion of the fatty acids into their methyl esters again did not affect the accuracy and precision of the weight determination. Thus, methyl *cis*-9-heptadecenoate may be used as an internal standard in the microdetermination of the weight of unsaturated lipids in plant material.

INTRODUCTION

The colorimetric reaction resulting from hydroxamic acid formation has generally been used until recently to quantitate ester groups of lipids[1]. At present the determination of the weight of small amounts of lipid is generally carried out by gas–liquid chromatography (GLC) with an internal standard[2–6]. In addition to high sensitivity and speed of analysis, the internal standard technique has the advantage of compensating for fatty acid losses that inevitably occur during the working-up of the biological material and the GLC determination itself.

However, to make full use of this advantage the fatty acid chosen as an internal

standard should be similar in its characteristics to the fatty acids of the sample under investigation and should not be present in the sample. Several saturated acids have been used as standards up to now[2–4,6] but most are unsuitable for our work where it is necessary to estimate the non-extractable phospholipids in soybean seeds in which unsaturated acids predominate. Therefore, we decided to use unsaturated *n-cis*-9-heptadecenoic acid as an internal standard. Until recently it was believed that this acid occurs in natural sources of lipids in trace amounts only[7,8]. However, the investigations of DYATLOVITSKAYA *et al.*[9] have shown that its content in the lipids of the *Candida* sp. yeasts grown on "odd" hydrocarbons may be as high as 50% of the total fatty acids. Thus, in view of rapid growth of the microbiological industry the yeasts might become a convenient source for the preparative isolation of heptadecenoate.

Up to now the amount of the fatty acids has been determined by using only single ratios of the sample/standard concentrations[2–6]. However, it could be argued that the accuracy of the weight analysis at the various ratios may be different since the response of many chromatographic detectors is known to be non-linear.

The present paper describes the calibration of the procedure for the gas chromatographic weight determination of fatty acids. *n*-Heptadecenoic acid isolated from the lipids of *Candida* sp. yeasts was used as an internal standard. The results obtained at different sample/standard ratios made it possible to determine the range of these ratios in which fair accuracy of the weight determination may be achieved and to estimate the precision of the latter.

EXPERIMENTAL

Reagents

Hexane and methanol were purified and dried as described previously[10]. Dodecane, ethanol, isopropanol, chloroform and hydrochloric acid were distilled[11,12], toluene and benzene were treated with H_2SO_4 and distilled over metallic Na. Dimethyldichlorosilane (DMDCS, technical grade) was dried for several days with anhydrous Na_2SO_4 and distilled, the 70° b.p. fraction being collected. Reagent grade chemicals—anhydrous aluminium oxide, argon, urea, silver nitrate, $NaIO_4$, $KMnO_4$, $NaHCO_3$, $Na_2S_2O_3 \cdot 5H_2O$, $K_2S_2O_3$, KOH, H_2SO_4, HNO_3, acetyl chloride, *tert.*-butanol, as well as Sudan Black B (Feinchemie K.-H., Kallies KG, G.F.R.), methyl caprylate and methyl caprate (California Corporation for Biochemical Research, Los Angeles, U.S.A., purity > 99.5% and 99.8%, respectively) were used without further purification. Adipic, azelaic and sebacic acids were obtained from the Institute of Chemistry of Natural Products, U.S.S.R. Academy of Sciences, and technical fat isolated from *Candida* sp. yeasts was supplied by the Moscow Branch of the All-Union Research Institute of Fats. Lipids extracted by cold hexane from ground soybean seeds were used to prepare fatty acid methyl esters[10].

Isolation of the unsaturated acids of yeast as their methyl esters

A flow diagram of the methyl heptadecenoate preparation is shown in Fig. 1: Nos. I–XIV refer to separate fractions while Nos. 1–12 indicate single operations during isolation and purification. At every stage of the preparative separation the fatty acid composition of the fractions was checked by analytical GLC[10]. Yeast fat I (4 g) was dissolved in a minimal quantity of hexane and transferred to a 2.4 × 9 cm

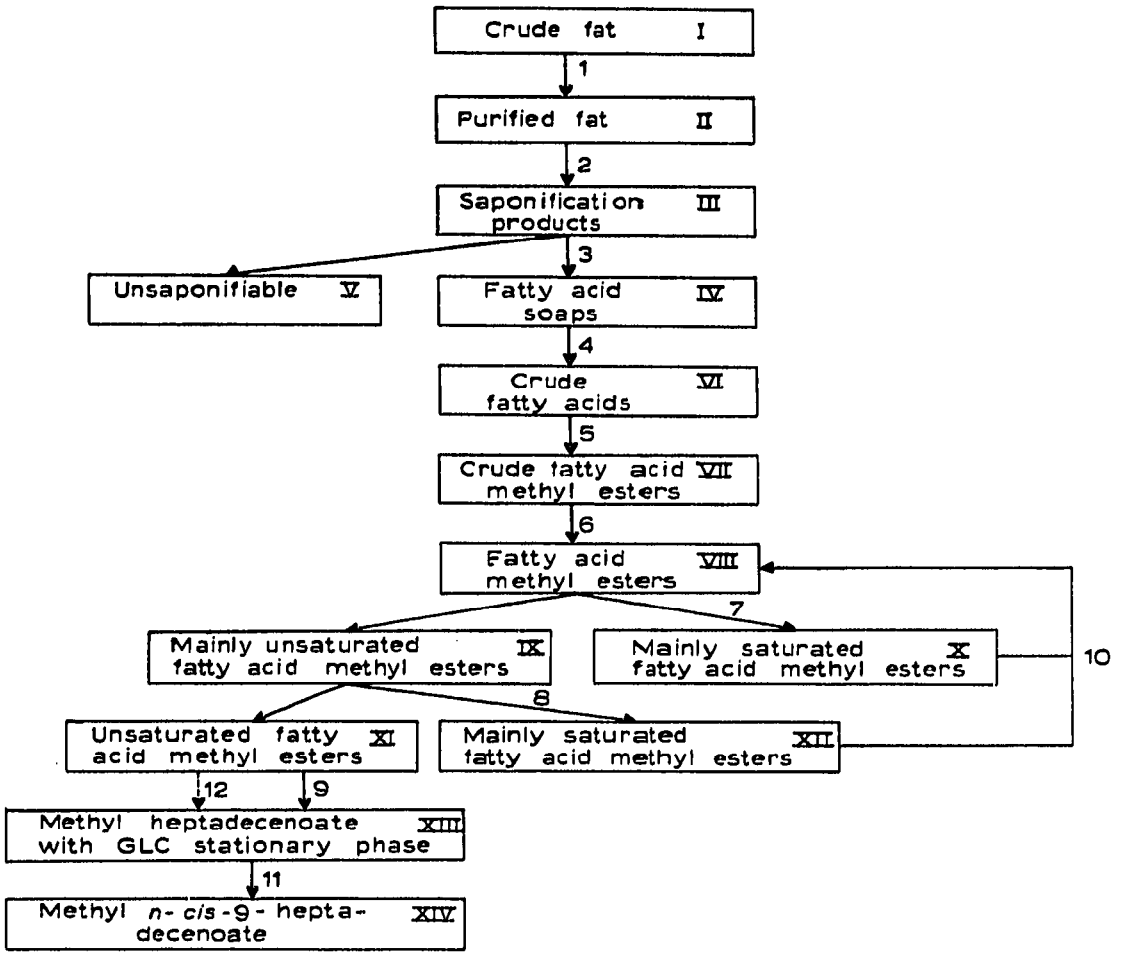

Fig. 1. Flow diagram of the preparative isolation of *n-cis*-9-heptadecenoic acid methyl ester. For designations of I to XIV and 1 to 12 see EXPERIMENTAL.

chromatographic column which was equipped with a No. 1 glass filter and contained 36 g of Al_2O_3. Triglycerides were eluted (1) from the column by 200 ml of hexane until the eluate became colorless. The partially purified fat II was saponified (2) in a solution of 500 mg of Na in 40 ml of ethanol for 1 h[10]. The ethanol was distilled off and the final traces were removed under vacuum at 50°. The saponification product III was transferred by 80 ml of H_2O to the continuous liquid–liquid extraction apparatus (total volume 100 ml) similar to that described previously[13] except that solvent vapors did not pass directly from the flask to the extraction chamber but to the upper end of the condenser of the apparatus. The aqueous phase was extracted with hexane for 13 h to remove most of color (3). Unsaponifiables V were discarded. Free fatty acids were regenerated from their soaps IV by H_2SO_4 acidification, and then extracted (4) with 10 × 7 ml of hexane and converted (5) to methyl esters VII[10,13] by 5 ml of methanol–acetyl chloride mixture (10:1). The esters VII were again purified (6) on Al_2O_3 as described above.

The purified esters VIII were dissolved in hexane to obtain a 10% (w/v) solution, an equal volume of saturated $AgNO_3$ solution in methanol was added and the mixture was shaken (7) in a separatory funnel. The upper phase containing mainly saturated esters X was separated and washed with water; hexane was distilled off, and the

residue was added (10) to fraction VIII during the next isolation. The mainly unsaturated esters of the lower phase IX were extracted with hexane after addition of excess water. The hexane was distilled off, and then a mixture of the esters IX (A g), urea (3.6 × A g), and methanol (16 × A ml) was heated until complete dissolution and allowed to stand overnight at room temperature. The supernatant fraction was filtered off on a Büchner funnel. The ester–urea complexes in both fractions obtained (XI and XII) were decomposed with an excess of hot aqueous HCl (20:1) and the esters were extracted with hexane (8). Mainly saturated esters XII as well as esters X were added (10) to fraction VIII.

Preparative GLC and storage of the preparation

The unsaturated esters XI were separated (9) on a preparative-scale gas chromatograph constructed in the Institute of Organic Chemistry, U.S.S.R. Academy of Sciences. The operating conditions were as follows: stationary phase, polyethylene glycol-2000 on Chromosorb P (20–30 mesh); column temperature, 200°; flash heater temperature, 220°; N_2 flow rate, 800 ml/min; column 20 m × 10–20 mm; HETP 1500; sample volume 1 ml. The isolated ester XIII was purified (11) by saponification (see above) from possible admixture of the stationary phase, unsaponifiables were repeatedly extracted with hexane in a flask with a side tube[14], and the heptadecenoic acid isolated after acidification was again converted into its methyl ester XIV.

The methyl heptadecenoate XIV so prepared was transferred to the storage ampul (Fig. 2) with a 5% (v/v) solution of methanol in benzene through the straight end 4. The joints were covered with a vacuum grease, and the stopcocks with female

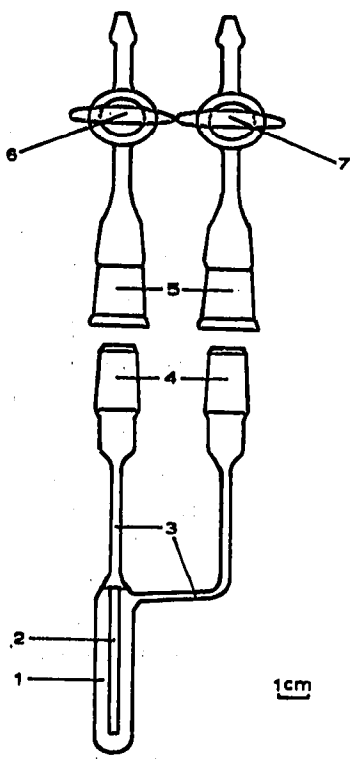

Fig. 2. Ampul for storage of the methyl heptadecenoate preparation. 1 = ampul; 2 = inert gas inlet tube; 3 = sealing points; 4 = male standard joint No. 14.5; 5 = female standard joint No. 14.5; 6 = gas inlet stopcock; 7 = outlet stopcock.

joints 5 were connected; the ampul was placed in an ice bath, and the argon flow was passed in through stopcock 6 at a rate of 10 ml/min and out through stopcock 7. 15 min later the ampul was immersed in solid carbon dioxide and the argon flow was maintained until the solution was frozen. Stopcocks 6 and 7 were then closed and the ampul was sealed, slightly opening the respective stopcocks immediately before sealing. The ampuled solution was stored in a frozen state at −5°.

Preparative partition chromatography

Preparation XIII can also be obtained (12) by partition chromatography of esters XI as their π-complexes with Ag ions[15]. The preparative isolation was performed using a 10% (v/v) dodecane solution and saturated $AgNO_3$ solution in 70% (v/v) aqueous methanol. Methyl heptadecenoate was eluted with hexane from unstained strips.

Determination of the physico-chemical characteristics of heptadecenoic acid and its methyl ester

The relative retention volume value was determined by GLC[10], the melting point was estimated according to TERENTYEV[16] and the refraction index n_D^{20} was measured at 20 ± 2° (ref. 17) on an IRF-22 refractometer.

Investigation of the structure of heptadecenoate

Oxidative degradation of the acid was performed by the method described previously[18,19]. The oxidation products were extracted with ether and traces of iodine were destroyed with a diluted $Na_2S_2O_3$ solution. Mono- and dicarboxylic acids resulting from the oxidation and pure adipic, azelaic and sebacic acids were converted into their methyl esters with a CH_3OH–CH_3COCl mixture (see above). Esters of mono- and dicarboxylic acids were separated by GLC[10], the column temperature being 125° and 150° and the carrier gas flow rate being 14 and 60 ml/min, respectively.

The IR spectrum of methyl heptadecenoate was obtained on a UR-10 spectrometer (Carl Zeiss, Jena, G.D.R.) under the following conditions: chart speed 150 cm^{-1}/min; scale of registration 12 mm/100 cm^{-1}; slit program 4; amplification 4.5; crystal support, potassium bromide.

Preparation of sample–standard calibration mixtures

A sample of soybean fatty acid methyl esters (40.8 mg) containing (as weight per cent of esters) palmitic (16.7), stearic (5.7), oleic (30.7), linoleic (44.7), linolenic (1.8) and arachidic (0.5) acids and free of any impurities was weighed to the fourth decimal point and dissolved in toluene in a calibrated[17] volumetric flask (nominal volume 50 ml). The same procedure was employed to prepare a solution of the standard preparation (17.7 mg), both solutions being obtained at the same room temperature (20–22°). Using a calibrated pipette[17] (nominal volume 5 ml), 12 mixtures having different sample/standard ratios were obtained (see *Calibration for the weight determination*). Toluene was distilled off *in vacuo*, the mixtures were dissolved in benzene and stored in ampuls as described above.

Preparation of the GLC column for calibration

Celite-545, 100–120 mesh, acid-washed (W. G. Pye and Co., Ltd., Great Britain)

was further purified with hydrochloric acid and fine particles of the support were removed with water; pure dry Celite was treated with 5% (v/v) solution of DMDCS in toluene[20]. The inside surface of the glass column and the glass yarn plug to be placed in the column above the solid support stood for 1 h in the DMDCS solution, were washed 4 times with benzene and stood for 1 h in methanol. The glass yarn was dried at 120° and the column in a current of air without heating. Polyethyleneglycol adipate (PEGA, 1 g) in a minimal volume of benzene was treated with an equal volume of the DMDCS solution; 10 min later the solvents and excess DMDCS were distilled off *in vacuo* at 60° and the stationary phase was dissolved in 80 ml of chloroform. The Celite was coated with PEGA according to a previously described method[10,21], $CHCl_3$ being removed in a rotary film evaporator (PVO-64, Mikrotechna n.p., Modřany, Czechoslovakia). The column was packed and conditioned as usual[10].

Calibration

The gas chromatograph equipped with an argon ionization detector was used[10]. The analysis of the prepared series of calibration mixtures was performed without interrupting the carrier gas flow and column heating. Three chromatograms were usually obtained for each calibration mixture; the composition of some mixtures was determined 10 times in succession to estimate the precision of the analysis. Only chromatograms having a maximum peak height between 50% and 100% of the recorder scale were used for peak area calculation which was performed by a triangulation technique.

Calculation of the true sample weight of fatty acid methyl esters as determined by GLC using an internal standard

The GLC analysis of one of the standard preparations showed that it contained 89.4% of methyl heptadecenoate; it also contained 2.3% of an impurity whose retention time was equal to that of methyl stearate. Therefore, the actual sample and standard weights (p and p_s, μg) in a given calibration mixture were

$$p = f \cdot a \left(P + \frac{P_s \cdot y}{100} \right) \text{ and } p_s = (f \cdot a_s \cdot P_s \cdot t)/100,$$

where

f = $v/5V$, the correction factor for the volumetric glassware calibration;
V = true volume of the volumetric flask (ml);
v = true volume of the volumetric pipette (ml);
P = sample weight in V ml of the sample solution (μg) ($P = 4.08 \cdot 10^4$ μg);
P_s = weight of the standard preparation in V ml of the standard solution (μg) ($P_s = 1.77 \cdot 10^4$ μg);
a = the nominal volume of the sample solution used to prepare a given mixture (ml);
a_s = the nominal volume of the standard solution used to prepare a given mixture (ml);
t = methyl heptadecenoate content in the standard preparation (weight %) ($t = 89.4\%$);
y = content of impurities whose retention time is equal to that of any sample peak in the standard preparation ($y = 2.3\%$).

The GLC determination of the weight of the fatty acids by the internal standard method is based on the assumption that there must be a direct linear relationship between the weights of the sample and standard, on the one hand, and the areas of total sample peaks and standard peak (S and s, mm^2), on the other. On the basis of this assumption, the weight of the fatty acid methyl esters sample as determined by GLC using an internal standard (p', μg) is

$$p' = (p_s \cdot S)/s,$$

where

$S = h_1 \cdot b_1 + h_2 \cdot b_2 + \ldots + h_N \cdot b_N$;
$s = h_s \cdot b_s$;
h_i = height of the i-th peak of the sample (mm);
b_i = width of the i-th peak of the sample (mm);
N = number of peaks of the sample;
h_s = height of the peak of the standard (mm);
b_s = width of the peak of the standard (mm).

As mentioned above, the response of many gas chromatographic detectors is non-linear. Therefore, the accuracy of the weight of fatty acid esters p' found should be assessed by the recovery (k, %) of the sample weight during the GLC determination of the latter value; $k = (p' \cdot 100)/p$. If it is assumed that theoretical recovery (k = 100%) is confined to only one value of the actual sample/standard weight ratio $Q = p/p_s$ or to a more or less narrow range of these values, then the true sample weight of fatty acid methyl esters as determined by GLC using an internal standard (p'_0, μg) in the region where k = 100% is $p'_{0,k=100\%} = p$, and beyond this region

$$p'_{0,k\neq 100\%} = p' \cdot \frac{100}{k} = \frac{f \cdot a_s \cdot P_s \cdot t\,(h_1 \cdot b_1 + h_2 \cdot b_2 + \ldots + h_N \cdot b_N)}{h_s \cdot b_s \cdot k}$$

In successive recovery determinations (see *Calibration*) yielding a number of k_i values for one or several calibration mixtures, the arithmetic mean of the recovery was calculated as $\bar{k} = \Sigma k_i/n$, where n is the number of measurements. In the $k \neq 100\%$ region $\bar{k}$ may be used for calculations of the corrected single recovery value $k'_i = 100\, k_i/\bar{k}$. The relative standard deviation S_{rel} for a number of $\bar{k}$ or k'_i values was determined by a previously described procedure (see Table II in ref. 17).

RESULTS AND DISCUSSION

Isolation and characteristics of heptadecenoic acid and its methyl ester

As mentioned above, the fatty acids previously used as internal standards in the weight determination of lipids[2–6] are sometimes inadequate for this work. Esters of normal acids with less than 16 carbon atoms in the chain (pentadecanoic, myristic) which have been used as standards owing to a low concentration of these acids in many living tissues[2] give very sharp peaks at isothermal column conditions. Measurement of the width of these peaks involves considerable error, and the appreciable volatility of the esters at room temperature may result in the loss of a standard during the working-up of the biological material before analysis. The disadvantages of margaric acid, which has frequently been used as a standard[3,4], are its high melting point (61°), difficulties involved in its purification and its occurrence in

TABLE I

COMPOSITION OF FATTY ACID METHYL ESTERS OF THE YEAST FAT VII AND THE FRACTION OF UNSATURATED ACID METHYL ESTERS XI (WEIGHT % OF ESTERS)

Fatty acid[a]	*Fraction VII*	*Fraction XI*
16:0	14.4	0.0
16:1	4.1	8.3
17:0	16.7	0.0
17:1	31.0	51.3
"18:0"	6.0	3.9
18:1	22.8	22.3
18:2	5.0	14.2

[a] 16:0 = palmitic; 16:1 = hexadecenoic; 17:0 = heptadecanoic; 17:1 = heptadecenoic; "18:0" = unidentified fatty acid: the relative retention volume of its methyl ester is close to that of methyl stearate, but according to the fractionation pattern this acid is similar to unsaturated acids; 18:1 = octadecenoic; 18:2 = octadecadienoic.

some natural fats. Branched-chain 2-methylalkanoic acids which have been employed as standards by NAPIER[5] can only be prepared by special synthesis, and so are not readily available.

The main requirements to be fulfilled by a fatty acid for use as an internal standard in the GLC weight determination of the residual lipids of soybean seeds are as follows: first of all it should not occur in this plant and moreover it should be as close as possible to the unsaturated acids predominantly occurring in soybean lipids in its physical characteristics—m.p., solubility, etc. Among the natural fatty acids *n-cis*-9-heptadecenoic acid meets these requirements best of all; during GLC on polar liquid phases the peak of its methyl ester is situated in the region between the peaks of methyl palmitate and methyl stearate usually not occupied by other esters.

The fat of *Candida* sp. yeasts used for the preparative isolation of heptadecenoic acid contains about 30% of this acid (Table I); the fractionation of Ag^+ coordination complexes and the urea precipitation result in an increase of the 17:1 content up to 50% and in complete removal of the saturated esters. The unsaturated fraction contains no methyl heptadecanoate (17:0) which would be difficult to separate from methyl heptadecenoate by preparative chromatography on a polar liquid phase because of the incomplete separation of their peaks. About 400 mg of 100% pure methyl heptadecenoate were isolated by GLC. The content of the major component in the preparation obtained by partition chromatography is about 98%, but the method itself is laborious and time-consuming.

TABLE II

CHARACTERISTICS OF *n-cis*-9-HEPTADECENOIC ACID AND ITS METHYL ESTER[a]

	V_R^{rel}		*Melting point (°C)*		n_D^{20}	
	Found	*Ref. 22*	*Found*	*Ref. 23*	*Found*	*Ref. 23*
Acid	—	—	14.0–14.1	8.7–9	1.4611	1.4594
Ester	3.12	3.12	4.1– 4.2	(−30.8)–(−30.3)	1.4533	1.4516

[a] V_R^{rel} = relative retention volume (V_R^{rel} of methyl myristate = 1.00).

To establish the structure of the isolated heptadecenoate its relative retention volume, melting point and refractive index were compared with the corresponding known data for natural and synthetic methyl *cis*-9-heptadecenoate and the free acid. As shown in Table II, the relative retention volume of methyl heptadecenoate is equal to that obtained for 17:1 from *Candida* lipids (see Fig. 1 in ref. 22). At the same time the melting point of synthetic *n-cis*-9-heptadecenoic acid and methyl heptadecenoate[23] are below those reported here. On the assumption that the ester melting point is −30.3° (Table II) we attempted to separate methyl heptadecenoate from other esters of fraction XI by crystallization in acetone at −78°; however, the 17:1 ester was always precipitated with methyl oleate. Found n_D^{20} values of both acid and ester were by 0.0017 higher than the data reported for the respective synthetic products.

Because of the above discrepancies, the position of the double bond in the chain of isolated methyl heptadecenoate was determined by oxidative degradation[18,19]. The retention volumes (V_R) of the methyl esters of standard mono- and dicarboxylic acids and heptadecenoate oxidation products are shown in Table III.

TABLE III

Methyl esters of	V_R, *ml Ar*
Caprylic acid	290
Capric acid	780
Adipic acid	265
Azelaic acid	969
Sebacic acid	1464
Oxidation products	294
	966

These data confirm the results of a previous investigation[9] according to which the double bond in the chain of *n*-heptadecenoic acid from *Candida* yeast is situated between the 9th and 10th carbon atoms. Higher acid and ester melting points found here might be supposed to be brought about by partial *cis, trans*-isomerization of the double bond during isolation and purification[23]. However, the absence of the *trans*-bond absorption band at 965 cm^{-1} in the IR spectrum of methyl heptadecenoate (Fig. 3) confirms the *cis*-configuration of the double bond of this ester reported previously[9].

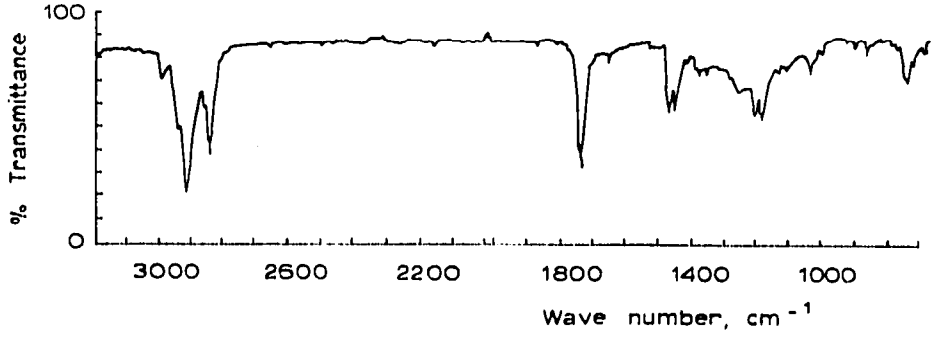

Fig. 3. IR spectrum of methyl *n-cis*-9-heptadecenoate.

Thus, the preparation isolated is the methyl ester of *n-cis*-9-heptadecenoic acid. The data available so far are insufficient to account for the differences in n_D^{20} and melting point between our preparation and the respective synthetic sample. It cannot be ruled out that this discrepancy might be associated with polymorphic differences in the crystals used in the m.p. measurements.

Calibration for the weight determination

The methyl heptadecenoate preparations obtained were used as an internal standard in the determination of the weight of total fatty acids. In practice it may become necessary to use the preparation of standard containing foreign impurities. Therefore, the calibration of the method was performed using one of the preparations containing 89.4% heptadecenoate, and corresponding correction factors t and y were introduced into the equations used to calculate the true sample weight (see EXPERIMENTAL).

The calibration was performed on a chromatographic column all components of which—inside surface, solid support and liquid phase—had been pretreated with dimethyldichlorosilane[20] to prevent selective adsorption of fatty acid methyl esters which could be affected by free silanol and hydroxyl groups of these components[24].

As shown in Fig. 4, the theoretical recovery of the sample weight ($\bar{k} \approx 100\%$) can be achieved only at $Q = 5.2$. Practical determination of the lipid weight requires, however, a more or less extensive range of Q ratios which should be characterized by equal or similar recovery values k. If this range were found, then by changing the weight of the added standard p_s it should be possible to obtain Q values for the lipid sample under investigation which will be within the limits of this range. There are horizontal sections of the curve (Fig. 4) at both $k < 100\%$ and $k > 100\%$. However, the $Q \leqslant 2.1$ region, owing to the small peak heights of the sample, does not provide sufficient precision for the determination of the weight and there is considerable scattering of the experimental points (Fig. 4). It was found that the range from

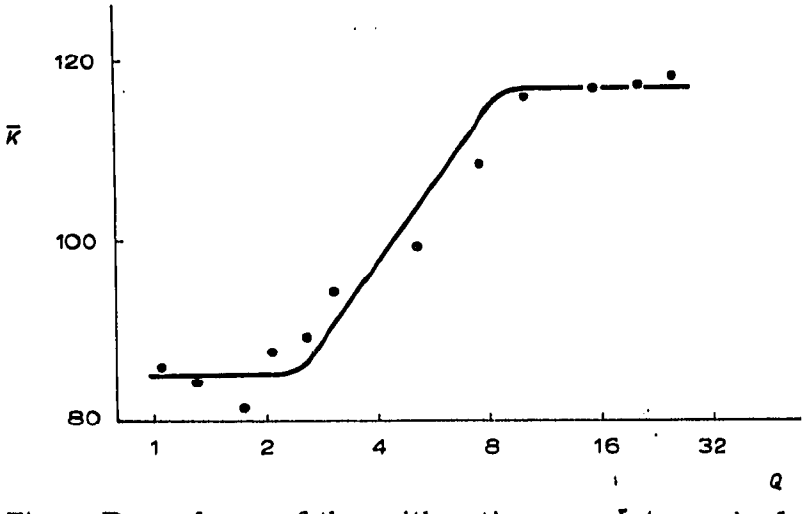

Fig. 4. Dependence of the arithmetic mean $\bar{k}$ ($n = 3$) of sample weight recovery as determined by GLC using an internal standard on the actual sample/standard weight ratio Q (Q values are plotted on the logarithmic scale).

$Q = 10.4$ to $Q = 25.9$ is most suitable for practical purposes since in this range the scattering does not exceed the error of replicate determinations of the same mixture (see below).

In the above range $\bar{k} = 115.8\%$ ($n = 12$). It should be pointed out that our preliminary data suggest that the absolute value of the correction coefficient $\bar{k}$ varies somewhat depending on the condition of the argon detector, even if basic process parameters remain unaltered[10]. Therefore, after a long time interval between experiments it is necessary to stabilize the detector by heating and to reascertain the $\bar{k}$ value using ready-made calibration mixtures. Over the whole working range $S_{rel} = \pm 3.7\%$ ($k'_i = 92.3$–105.5%); an increase in the $\bar{k}$ value by several unit per cent does not change the S_{rel} value.

In order to assess the comparative precision of the determination of the weights in the selected range, the S_{rel} values for the mixtures with $Q = 15.5$ and 25.9 were compared with the S_{rel} value at $Q = 5.2$ ($\bar{k} \approx 100\%$). The S_{rel} values found were $\pm 1.5\%$, $\pm 2.8\%$ and $\pm 3.1\%$, respectively ($n = 10$ in all cases). It can be seen that there is not much difference between the errors in the determination of the weight at arbitrary points of the range, at the point where $\bar{k} \approx 100\%$ and in the whole working range ($S_{rel} = \pm 3.7\%$). It can be concluded that this range provides not only fair accuracy but also sufficient precision for the weight analysis.

In a determination of the weight of the lipids in living tissues, the standard is usually added to the untreated plant material which is subsequently subjected to saponification. To assess the effect of this treatment on the accuracy and precision of the weight determination of the fatty acid methyl esters, the calibration mixture of the esters with $Q = 25.9$ was saponified, unsaponifiables were repeatedly extracted and fatty acids were again converted to their methyl esters. Before and after saponification mean arithmetic values of k'_i ($n = 10$) were 101.6 and 101.5%, respectively; the error of analysis did not change due to the saponification ($S_{rel} = \pm 2.8\%$).

REFERENCES

1 G. B. ANSELL AND J. N. HAWTHORNE, *Phospholipids*, Elsevier, Amsterdam, 1964, p. 57.
2 M. L. BLANK, B. VERDINO AND O. S. PRIVETT, *J. Am. Oil Chemists' Soc.*, 42 (1965) 87.
3 I. HORNSTEIN, J. A. ALFORD, L. E. ELLIOTT AND P. F. CROWE, *Anal. Chem.*, 32 (1960) 540.
4 J. TINOCO, A. SHANNON AND P. MILJANICH, *Anal. Biochem.*, 3 (1962) 514.
5 E. A. NAPIER, *Anal. Chem.*, 35 (1963) 1294.
6 W. L. ZIELINSKI AND I. R. VIRGINIA, *J. Am. Oil Chemists' Soc.*, 41 (1964) 249.
7 M. J. CHISHOLM AND C. Y. HOPKINS, *Can. J. Chem.*, 35 (1957) 1434.
8 F. B. SHORLAND AND A. S. JESSOP, *Nature*, 176 (1955) 737.
9 E. V. DYATLOVITSKAYA, K. P. GRESHNYKH AND L. D. BERGELSON, *Prikl. Biokhim. Mikrobiol.*, 1 (1965) 613.
10 A. G. VERESHCHAGIN, S. V. SKVORTSOVA AND N. I. ISKHAKOV, *Biokhimiya*, 28 (1963) 868.
11 YU. K. YURIEV, *Prakticheskiye raboty po organicheskoi khimii*, Moscow University Publishing House, Moscow, 1961.
12 P. P. KOROSTELEV, *Prigotovleniye rastvorov dlya khimikoanaliticheskikh rabot*, "Nauka" Publishing House, Moscow, 1964.
13 A. G. VERESHCHAGIN AND G. L. KLYACHKO-GURVICH, *Biokhimiya*, 30 (1965) 543.
14 M. KATES, *J. Lipid Res.*, 5 (1964) 132.
15 A. G. VERESHCHAGIN, *J. Chromatog.*, 17 (1965) 382.
16 S. VEIBEL, *Identifikatsiya organicheskikh soyedinenii*, Foreign Literature Publishing House, Moscow, 1957, p. 316.
17 A. V. ZHUKOV AND A. G. VERESHCHAGIN, *J. Lipid Res.*, 10 (1969) 711.
18 L. CHANG AND C. SWEELY, *J. Lipid Res.*, 3 (1962) 170.
19 K. P. GRESHNYKH, Lipids of yeasts grown on *n*-alkanes, *Thesis*, Institute of Chemistry of Natural Products, Academy of Sciences, Moscow, 1968.

20 W. R. SUPINA, R. S. HENLY AND R. F. KRUPPA, *J. Am. Oil Chemists' Soc.*, 43 (1966) 202A.
21 A. G. VERESHCHAGIN, in H. G. STRUPPE (Editor), *Gas-Chromatographic 1965*, Akademie-Verlag, Berlin, 1965.
22 E. V. DYATLOVITSKAYA, K. P. GRESHNYKH AND L. D. BERGELSON, *Biokhimiya*, 33 (1968) 83.
23 G. GRIMMER AND J. KRACHT, *Chem. Ber.*, 96 (1963) 3370.
24 A. SEHER, *Fette, Seifen, Anstrichmittel*, 68 (1966) 255.