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A CONVENIENT and ECONOMIC METHOD for THE PREPARATION of PHEROMONES

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Abstract- The preparation of (Z)-9-tetradecenoic acid from beef tallow was assessed. Over one kilogram of this acid was obtained,in high purity, by a stepwise procedure involving fractional distillation, low temperature crystallization from acetone and fractional crystallization. Confirmation of the structure of the isolated acid methyl ester was achieved by reductive ozonolysis and IR spectra.This ester was utilized as starting material for the preparation of (Z)-9-tetradecenol an expected sex pheromone of the fall army worm. Other pheromones; (Z)-9-tetradecenyl acetate and (Z)-9-tetradecenyl aldehyde were prepared. The three compounds are now being tested in the field as sex attractants.

I N T R O D U C T I O N

Many aliphatic compounds having 12,14,or 16 carbon atoms and one or two double bonds can function as "chemical messengers" between the different sexes of the same insect species (1,2). However,contrary of the popular impression, insect sex attractants are not necessarly species-specific,sometimes not even genous specific or family specific (2). The concentrations required of these so-called "pheromones"(3)are extremly small(4). By oversaturating the mating area of a certain insect species with the respective pheromone,it is possible to inhibit olfactory communication between these insects(5).

Moreover, by the use of pheromones, males or females of a certain insect species may be lured into survey traps, where they can be treated with insecticides or killed by any other means(6). Hence, pheromones can be used for the biological control of insect pests without contaminating the environment with rather high concentrations of chemicals that may be harmful to man and domestic animals.

As a rule, the preparation of sex attractants by chemical synthesis is tedious, mainly because of the difficulties encountered in separating the geometrical isomers formed(7). As (Z)-9-tetradecenol, the sex attractant of the fall army worm(Laphygma frugiperda), can be obtained by a Wittig synthesis(8), but this compound must be separated from its (E)-isomer by chromatography(7).

It has been observed in this laboratory that beef tallow and fats from slaughtering wastes are excellent and extremly inexpensive raw materials for the isolation of mono-unsaturated fatty acids having 12, 14 and 16 carbon atoms. The present communication presents a process of isolation of (Z)-9-tetradecenoic acid in pure form from beef tallow that contains only between 0.2 and 0.5% of tetradecenoic acid. The method of separation selected was based on the difference in solubility of fatty acids or their urea adducts when present as mixtures in acetone or methanol respectively. In addition, the preparation of the expected pheromones ;(Z)-9-tetradecenol, (Z)-9-tetradecenyl acetate and (Z)-9-tetradecenyl aldehyde from the corresponding fatty acid is also reported.

E X P E R I M E N T A L

Instrumentation- A superspeed refrigerating centrifuge[1], a micro ozonizer[2](10), an infrared spectrophotometer[3]and a gas chromatograph (GC)[4]equipped with ; needle valves and a flame ionization detector, a 10 ft stainless steal capillary column packed with 10% ethylene glycol succinate silicone(EGSSX) on 100-120 mesh.,temperature employed was ; injection port 250°C, detector oven 250°C, column 180°C for isothermic determination and 40-180°C for programming,nitrogen flow through the column was 15 ml N_2/min.

Materials- About 100 kg of the distilled fatty acids from beef tallow were provided[5]. Reference standards for GC and reductive ozonolysis were commercially available[6]. All other reagents and chemicals were analytically pure.

Methods- 1- Fractiomal crystallization of fatty acids: Series of 10%solutions(W/V) of the selected fraction in acetone or in saturated solution of urea in methanol were prepared. The acetone solutions were cooled to +5,-18,and-36°c,whereas, the other solutions in urea were kept at +20,+5,and-18°C. Supernatent liquides were separated by filtration or centrif-

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[1]SORVALL

[2]SUPELCO,INC.Supelco Park,Pansylvania USA.

[3]Zeis , Germany.

[4]Perkin Elmer , model F 7 USA.

[5]Henkel and Cie"German chemical company,Düsseldorf.

[6]Hormel Foundation,USA.

ugation[7] with essential ultra cooling systems.

2- Preparation of concentrate : The supernatant solutions collected after crystallization from acetone at -36°C were concentrated in a rotary evaporator[8] to one third their volume then recooled at -36°C and the precipetates formed overnight were collected by centrifugation.

3- Preparation of methyl ester- Methyl esters needed for GC, fractional crystallization , urea adduction and fractional distillation were prepared by the method of Sietz(9), where benzene, methanol and sulfuric acid were employed in the ratio of 10:84: :6 respectively.

4- Reductive ozonolysis- Analytical grade pentane was used for preparing the sample solution, 2.5ug/µl pentane,(11). In 100µl sample solution, cooled at ca. -60°C in methanol dry ice bath, was passed ozone 10ml/min. Time needed for complete ozonolysis[9] was in the range of 1.5-2.0 min. Excess ozone in the solution was blowed out by stream of nitrogen. Residue of the ozonoid was redissolved in pure carbon disulfide then ca. 5µg of this solution was injected directly into GC for the analysis of the fragments formed. Programmed GC was suitable for this purpose.

5- Preparation of derivatives- a- Alcohol . The preparation from tetradecenoic acid was achieved by reduction of the methyl ester with lithium aluminum hydride in dry diethyl ether. The product, in etherial solution, was worked up in the usual way(12).

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[7] 10,000 rpm.

[8] Under stream of nitrogen.

[9] Detected by acidified starch potassium iodide paper.

2- Acetate : The preparation of the acetate was performed by reduction of the methyl ester with lithium aluminium hydride in diethyl ether followed by acetylating decomposition of the resulting lithium alumino complex with acetic acid(13).

3- Aldehyde :The preparation of aldehyde recommended the preformation of the mesylate. The reaction of methanesulphonyl chloride with tetradecenyl alcohol in absolute pyridine yielded the tetradecenyl methanesulphonate(mesylate)(14). Oxidation of the mesylate with dimethyl sulphoxide led to the formation of tetradecenyl aldehyde(15).

Analysis of the isolated acid ester and the prepared derivatives: The purity of the isolated ester and its derivatives was assessed by thin-layer chromatography and gas chromatography. The presence of impurities in the products could be detected by chromatography on thin layers of silica gel using ether:hexan in the ratio of 1:4 for developing the ester,alcohol and aldehyde,while benzene was used as the developing solvent for the mesylate followed by charring with chromic-sulfuric acid solution.

RESULTS and DISCUSSION

Trials for isolation of the precursors of pheromones were performed on beef tallow that contains (Z)-9-tetradecenoic acid,the precursor of the pheromone of the fall army worm.

The German chemical company fractionated the fatty acids of beef tallow in two steps. The first distillation yielded a mixture containing 3.5% of tetradecenoic acid(I). The second

distillation yielded twenty seven fractions in which the content of (I) was different (Table I). Portions of the latter fractions were used in our laboratory for the further enrichment of the desired acid.

Table I- Fractionation of Fatty Acids from Beef Tallow.

In Table II are given the results of experiments carried out to find optimum conditions for the enrichment of (I) or its ester, methyl tetradecenoate (II) by crystallization from acetone and by the formation of urea adducts . The material worked with contained 10.3% of (I) or (II). The concentration of the acid or the ester in the filtrate was determined by GC at 180°c, isotherm .

Table II- Enrichment of Tetradecenoic Acid and Methyl Tetradecenoate by Crystallization from Acetone and Formation of Urea Adducts

It is evident from the data given that best results were obtained by crystallization of the fatty acid mixture from acetone at -36°C. Therefore, several fractions of the second distillation were further fractionated by crystallization from acetone at -36°C. Thus, 10 kg of a mixture of fatty acids containing 28.6% of (I) were obtained. This material was used for the preparation of the concentrate of (I), that yielded 2.2 kg of a supernatent solution,which contained 50.5 % of this acid. After esterification, the methyl esters of these fatty acids were distilled at reduced pressure. The distillation

provided 1.5 kg of fractions, that contained from 65 to 80 % of (II). Recrystallization of these fractions without the use of a solvent, yielded 0.8 kg of a concentrate containing over 90 % of methyl tetradecenoate (Table III).

Table III- Preparation ofConcentrate of Methyl Tetradecenoate.

Since attractancy of insect attractants is dependent upon structure, and a single change in the stereochemistry of the double bond may greatly decrease the biological activity (4), it was essential that this candidate, methyl tetradecenoate, be of known isomeric composition and purity, In order to determine this structure in the concentrate, 0.1 mg of this preparation was subjected to reductive ozonolysis and analyzed the fragments formed by GC. The methyl esters of palmitoleic and oleic acids were similarly treated so as to be used for comparison. The chromatograms in Figure I and Figure II showed that, ozonolysis of the methyl esters of mono-unsaturated fatty acids having 14,16 and 18 carbon atoms yielded a C_9-aldester and a C_5-aldehyde in the case of the isolated methyl tetradecenoate, a C_9-aldester and a C_7-aldehyde in the case of methyl palmitoleate and a C_9-aldester and a C_9-aldehyde in the case of methyl oleate. Consequently, the ester in the concentrate consisted almost of the 9-isomer. Positional isomers, namely, the methyl esters of tetradecenoic acids having the double bond in 11 or 13 positions were in proportions of about 1 %, each, or less .

NMR spectrum of the isolated methyl ester in carbon tetra-

chloride (Figure III) showed a pattern similar to that for methyl oleate (I6), where the observed values correspond to the following: olefinic = 4.72, OCH_3 = 6.38, CH_2CO = 7.83,b, umc, CH_3- C= 9.I2,t and CH_2 - (non allylic) = 8.72,b. Therefore, this NMR spectrum confermed not only the structure of the isolated ester, but also its(Z) - isomeric form by its similarity to methyl oleate. Moreover, infrared spectroscopy, the standard method for analysis of trans content(I7), as well as argentation chromatography on thin layers of silica gel impregnated with 20% silver nitrate proved the absence of the (E) - isomer. Hence, it is concluded that the methyl ester of the mono-unsaturated fatty acid in the concentrate consisted mainly of methyl (Z) - 9- tetradecenoate.

Pure methyl(Z)-9-tetradecenoate was easily obtained from the concentrate by coloumn chromatography on Florisil containing silver nitrate. This adsorbent has high capacity and is,therefore, applicable on a semi-preparative scale. From 50 gm of concentrate was isolated 42 gm of pure methyl tetradecenoate.

Aliquots of the concentrate as well as the pure methyl ester were used for the preparation of several suspected pheromones; the tetradecenol, tetradecenyl acetate and tetradecenyl aldehyde.

The alcohol and acetate derivatives were obtained in quantitative yield, while the aldehyde was obtained in an overall yield of about 55%. The three derivatives, (Z)-9-tetradecenol,(Z)-9-tetradecenyl acetate and (Z) -9-tetradecenyl aldehyde had properties in agreement with those described in the leterature (I8). These derivatives are now being tested in the field by entomologists in Canada, Germany and the United States.

We are particularly interested in fiding out whether pure compounds are required or whether substances prepared from the concentrate would be good enough to be used as sex attractants in the biological control of insect pests.

A CONVENIENT AND ECONOMIC METHOD FOR THE PREPARATION

From the presented method for the isolation of pheromone precursors and preparation of the expected pheromones, it is evident that, the method is convenient and economic.

When one starts with 100 kg of beef tallow then apply the previous procedure, he can obtain ca, 0.5 kg of tetradecenoic acid (Table IV). This is rather inexpensive when compared with the milligrams isolated from insects (19) and if prepared by the tedious scheme of synthesis followed by separation of the geometrical isomers formed(4,18).

Table I- Fractionation of Fatty Acids from Beef Tallow

Total fatty acids
750 kg
(0.2 % Tetradecenoic acid)

↓ First distillation

Forerun of distillation
107 kg
(3.5 % Tetradecenoic acid)

↓ Second distillation

Fractions 1-10, 34.5 kg
(0-5.5 % Tetradecenoic acid)

Fractions 11-27, 60.3 kg
(10.3-15.9 % Tetradecenoic acid)

Residue, 7.0kg
(4.5 % Tetradecenoic acid)

Table II- Enrichment of Tetradecenoic Acid and Methyl Tetradecenoate by Crystallization from Acetone and Formation of Urea Adducts

Starting material: 10.3 % tetradecenoic acid or methyl tetradecenoate		Composition of filtrate (% tetradecenoic acid or methyl tetradecenoate)
Enrichment of tetradecenoic acid		
by crystallization from 10 % solution in acetone	+ 5°C	10.3
	-18°C	16.8
	-36°C	28.6
through formation of urea adducts from 10 % solution in methanol	+20°C	13.6
	+ 5°C	15.7
	-18°C	18.1
Enrichment of methyl tetradecenoate		
by crystallization from 10 % solution in acetone	+ 5°C	10.3
	-18°C	10.3
	-36°C	15.0
through formation of urea adducts from 10 % solution in methanol	+20°C	10.3
	+ 5°C	13,0
	-18°C	15

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Table III- Preparation of Concentrate of Methyl Tetradecenoate

Solution of mixture of fatty acids, 10 kg, in acetone (30 %, w/v)
(28.6 % tetradecenoic acid)

↓ Cooling to -36°C followed by centrifugation

Supernatent
2.2 kg
(50.5 % tetradecenoic acid)

↓ Esterification followed by distillation of methyl esters at 12 mm

Fractions of methyl esters
1.5 kg
(65-80 % methyl tetradecenoate)

↓ Cooling to -36°C followed by centrifugation

Supernatent
Concentrate
0.8 kg
(90 % methyl tetradecenoate)

Table IV- Isolation of Tetradecenoic Acid from Beef Tallow

Step	Starting[1] (total F.A.)	First distill.	Second distill.	Enrichment	Concent-ration	Distill. (12mm)	Cooling -36 C	Florisil CC[2]
Wt-kg	100	14.3	10.7	7.1	1.6	1.02	0.55	0.46
% I[3] or II	0.2	3.5	10.3	28.6	50.5	65-80	90	99

[1] F.A. : Fatty Acids.

[2] CC : Column Chromatography.

[3] I or II : Tetradecenoic Acid or Methyl tetradecenoate respectively.

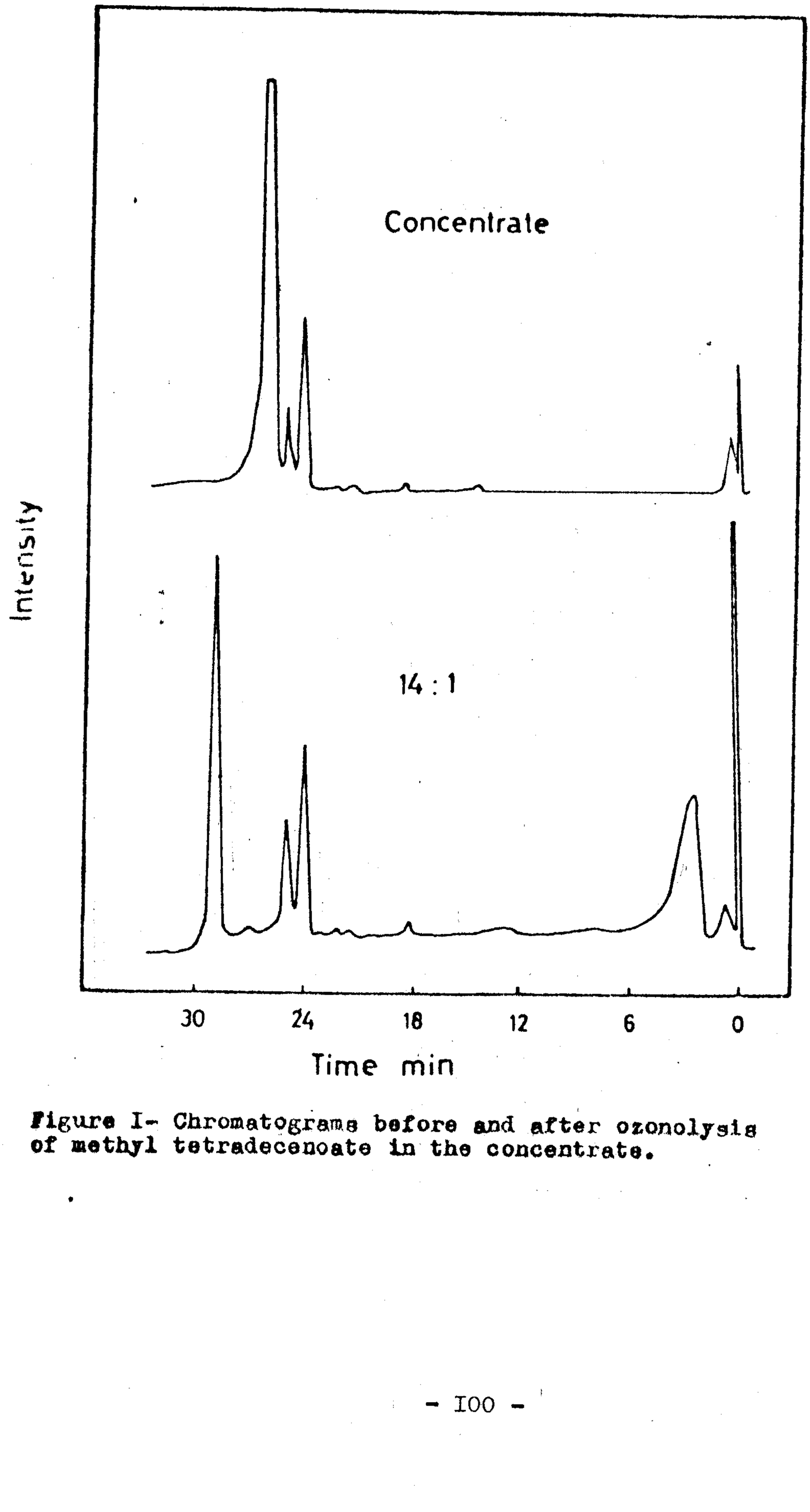

Figure I- Chromatograms before and after ozonolysis of methyl tetradecenoate in the concentrate.

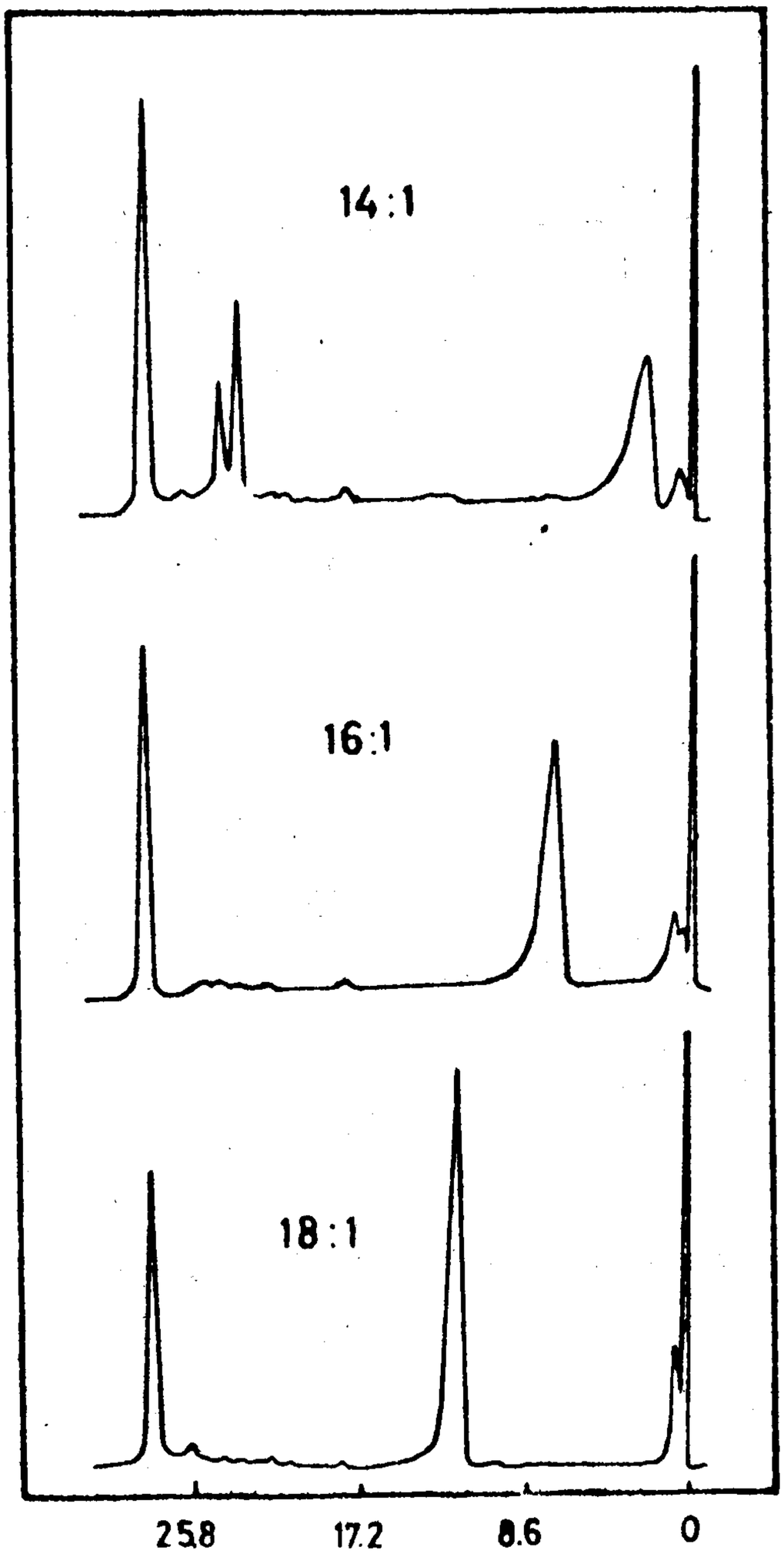

Figure II- Chromatograms after ozonolysis of the methyl esters of:tetradecenoic(14:1), palmitoleic(16:1) and oleic(18:1) acids.

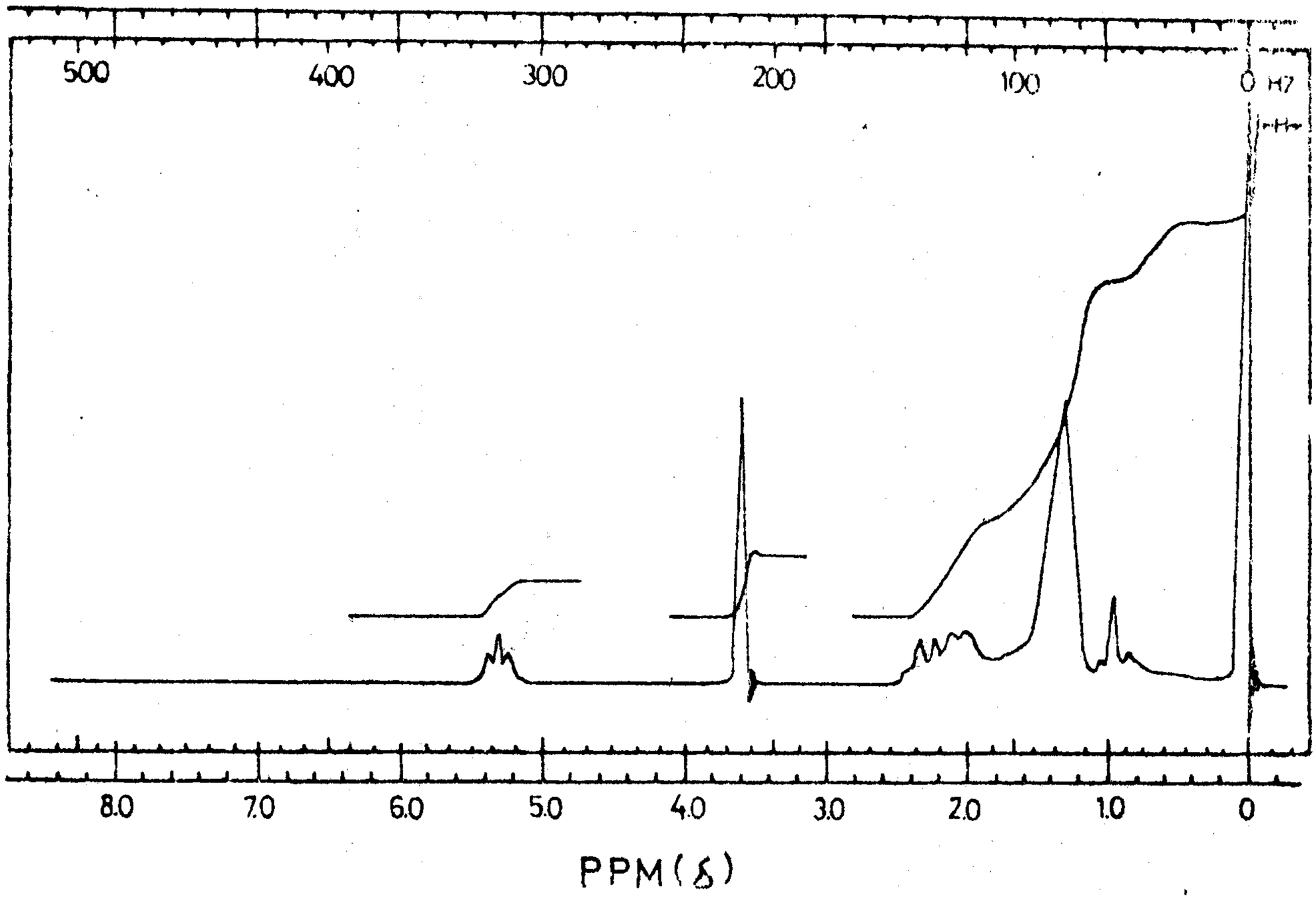

Figure III- NMR spectrum of methyl tetradecenoate.

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التحضير الاقتصادى والعملى لبعض جاذبات الحشـــــرات

نوال على الرباط ـ وهلموت كـــارل مانجولـــد

المـركز الفيـدرالى لابحـاث الدهون ـ معـهد الكيمياء الحيوية والتكنولوجيا

مونســـتر ـ المانيـــا الغربيـــة

ان جاذبات الحشرات ـ الفيرمونـات ـ تتكون غالبا من جزيئات غير حلقية يتراوح عـدد ذرات الكربـون فيها بين ١٢ ـ ١٤ر١٦ ذرة ، وتحتوى على رابطة مزدوجـة واحـــــدة او اثنتـان • تأثير هـذه المركبات لا يعتمـد فقط على مكان الرابطة المزدوجة في الجـزىء بـل وعلى نـوع التنـاظر للذرات المتصـلة بالرابطـة المزدوجـة • ولقـد اثبت عمليـــا ان المركبات ذات التناظر المتجـاور اكثـر فاعليـة من الاخـرى • الحصـول على هـــذه المركبـات مـن الحشرات المفـرزة لهـا ممكن ولكن بنسب ضئيـله جـدا • اما التحضيـر الكيميائـى لهـذه المركبات فليـس بالسـهـل وخصوصا عند الالتجـاء للكروماتوجرافيا لفصـل المركبات المتناظـرة بعضهـا عن بعض•

ولقد لوحظ ان الشحم البقرى ونفايات الذبائح تحتوى على احماض دهنية غير مشبعة لهـا نفس التكوين السابق ذكره • ولهذا فلقـد تطرق البحث الحالى الى فصل حمض البرستأولبيـــك ـ رائد فيرومـــون بعض الحشرات مثل العناكب من دهـــن البقر الحاوى له بنسبة ٢ر٠٠٪ •

ولقد تـم بالفعل فصـل حوالى كيلوجرام من هذا الحمض مستخدمين طريقة الفصل بالتقطير التجـزىء ثم التبـريد والفصـل من الاسيتـون ثم التيلر التجزيء بالتبريد بعد التقطيـــــر التجـــزيء ـ وللتأكـد من هويـة الحامض المفصول وموضع الرابطة المزدوجة استعملت طريقـــة الاخـتزال الأوزونى ثم تحليل العظام بالغاز كروماتوجرافيا • أما نوع التناظر فتأكد لدينا انـــه متجـاور وذلك بالاشعة دون الحمراء من هذه التحاليل تبين لنا ان الحمض الذى حصلنا عليه هـو حمض الميرستأولبيك ذو التناظر المتجاور من هذا الحمض ثم تخليق مشتقات بسيطة يرجى لها فاعلية كجاذبـة للحشرات الضارة التى يمكن ابادتها بعيـدا عن المناطق الحيوية • ولقد ارسلت هذه المركبات وهـى الكحول والالدهيـد وخـلات حمض الميرستاولبيك الى المتخصصين بالمانيـــا الغربيـــة ـ كنـــدا والولايات المتحـدة الامريكية للتأكـد من فاعليتها البيولوجيـة •