

# The Preparation, Gas Liquid Chromatography, and Some Physical and Chemical Properties of Certain Higher Aliphatic 2,4-Diketones — A New Lipid Class

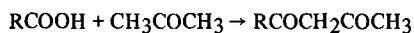
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## ABSTRACT

Fourteen saturated aliphatic 2,4-diketones, ranging in chain length from C<sub>7</sub> to C<sub>25</sub>, and one unsaturated compound,  $\Delta^{12}$ -2,4-heneicosenedione, were prepared by Claisen acylations of the appropriate methyl ketones, and their gas chromatographic behavior was studied. Mixtures of these diketones were resolved on columns containing UCW98 or Carbowax 20M stationary phases. Their methoxime and 2,4-dinitrophenylsemicarbazone derivatives were resolved on OV-17 and OV-1 stationary phases, respectively. Since higher homologous aliphatic 2,4-diketones have been found in mammalian tissues, they constitute a new lipid class.

## INTRODUCTION

A group of homologous aliphatic 2,4-diketones with chain lengths ranging from C<sub>13</sub> to C<sub>25</sub> has been found widely distributed in mammalian tissues. These compounds, which comprise a new lipid class, are apparently derived in vivo from the naturally occurring fatty acids through the condensation of a molecule of fatty acid with a molecule of acetone:



where R = CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub> and n = 8—20

It appears that unsaturated chains may also occur; however, the saturated compounds, with n even, are the more abundant. The inhibition of the action of histamine on smooth muscle by tissue extracts (1-4) has been attributed by us to the presence of these 2,4-diketones in such extracts. Details concerning the isolation of this 2,4-diketone fraction from tissues, its gas liquid chromatographic resolution, and the confirmatory identification of individual components will be presented in another publication. This communication deals with the synthesis of a

number of 2,4-diketones which were required for reference purposes and for pharmacological investigations, their gas liquid chromatographic behavior, and with some physical and chemical properties of these compounds.

Syntheses of various homologous saturated 2,4-diketones have been reported in the literature; they were usually prepared by a Claisen condensation between the appropriate methyl ketone and ethyl acetate in the presence of sodium (5-8), sodamide (9), or sodium hydride (10), or, alternatively, by the condensation of a fatty acid ethyl ester with acetone in the presence of one of these condensing agents. Several 2,4-diketones have been synthesized as well by the reaction of acetoacetyl chloride with alkylmagnesium bromides (8).

For this present investigation, a few 2,4-diketones, up to 2,4-tetradecanedione, were conveniently prepared by a Claisen condensation between the appropriate commercially available methyl ketone and acetone in anhydrous diethyl ether, with sodium hydride as the condensing agent (10); for the preparation of higher homologues, the required methyl ketones were synthesized by the acylation of ethoxymagnesiummalonic ester with the appropriate fatty acid chloride, followed by acidolysis of the resulting acylmalonic ester to the desired methyl ketone (11,12).

The 2,4-diketones, which were thus synthesized, were amenable to gas liquid chromatography without prior derivatization. Of the several stationary phases, including OV-1, PPE-20, UCW98, and Carbowax 20M, which were investigated, UCW98 was found to be satisfactory. Most of our work was done with columns packed with 10% UCW98 on Diatoport S or Chromosorb W-AW (DMCS) which were operated isothermally.

2,4-Dinitrophenylhydrazones and -semicarbazones (13,14) of several 2,4-diketones were prepared. The former were viscous oils and were not investigated further. The 2,4-dinitrophenylsemicarbazones were chromatographed on a 3% OV-1 column operated at 200 C. Methoxime derivatives (15,16) of 2,4-diketones were resolved on a 3% OV-17 column at 230 C.

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## MATERIALS AND METHODS

### Sodium Hydride

Sodium hydride, 50% in oil, was purchased from British Drug Houses. Just prior to use, the oil was removed by washing the weighed sample of NaH with petroleum ether (30 C-60 C) in the reaction flask.

### Methyl Ketones

2-Pentanone, 2-heptanone, 2-octanone, 2-undecanone were purchased from J.T. Baker Chemical Co. and Anachemia Chemicals Ltd., Montreal and were of practical or laboratory grade.

### Fatty Acids

These were of laboratory grade. Pelargonic acid was synthesized from 1-bromooctane and sodium cyanide.

### Florisil

Florisil (60-100 mesh), Floridin Company, Tallahassee, FL, was treated with 1:1 hydrochloric acid, washed repeatedly with distilled water, and then was dried at 120 C and activated at 300 C overnight.

### Gas Liquid Chromatography (GLC)

A Hewlett-Packard Model 5750 gas chromatograph, with dual columns and dual flame ionization detectors, was employed. For the chromatography of 2,4-diketones, a 6 ft x 1/8 in. stainless steel column, packed with 10% UCW98 on Diatoport S or Chromosorb W-AW (DMCS), was used. It was operated isothermally at 200 C, 230 C, or 240 C. Injection port and FID temperatures were 240 C and 260 C, respectively, and the helium flow rate was 30 ml/min. Mixtures of 2,4-diketones were separated also with a 6 ft x 1/8 in. stainless steel column packed with 4% Carbowax 20M on Diatoport S, and operated at 210 C or at 220 C. For the methoximes, a 6 ft x 1/8 in. stainless steel column, containing 3% OV-17 on Chromosorb W, was employed. The 2,4-dinitrophenylsemicarbazones were separated on a 30 in. x 1/4 in. glass column packed with 3% OV-1 on Gas Chrom Q.

### Ultraviolet Spectrophotometry

A Beckman DK-2 Ratio-Recording Spectrophotometer was employed.

### Infrared Spectrophotometry

Infrared spectra were obtained with a Perkin-Elmer Infracord. Samples were mounted as liquid films between sodium chloride discs.

### Melting Point Determinations

A Kofler micro-melting point apparatus, attached to the stage of a microscope, was employed. Eutectic melting points of mixtures of several crystalline 2,4-diketones with azobenzene have been recorded as well.

### Syntheses of 2,4-Diketones

The following 2,4-diketones were synthesized by the condensation of the appropriate methyl ketone with ethyl acetate in the presence of sodium hydride (10): 2,4-heptanedione, 2,4-nonanedione, 2,4-decanedione, 2,4-tridecanedione, and 2,4-tetradecanedione. 2,4-Octadecanedione was prepared from 2-hexadecanone which was obtained by the oxidation of 2-hexadecanol. The diketones were purified via their copper chelates, and the lower members were distilled in vacuo.

The following 2,4-diketones were synthesized from methyl ketones which were prepared from the acid chlorides of the appropriate fatty acids (11,12): 2,4-dodecanedione, 2,4-pentadecanedione, 2,4-heptadecanedione, 2,4-nonadecanedione, 2,4-eicosanedione, 2,4-heneicosanedione,  $\Delta^{12}$ -2,4-heneicosanedione, 2,4-tricosanedione, and 2,4-pentacosanedione.

Three typical syntheses are summarized below. Full details regarding the preparation of the compounds mentioned above will be provided to interested parties on request.

### Synthesis of 2,4-Nonadecanedione

2-Heptadecanone was prepared from palmitoyl chloride and ethoxymagnesiummalonic ester (11,12). The 2-heptadecanone was distilled at ca. 0.1 mm Hg in a Hickman still. The yield, based on the palmitoyl chloride, was 58%. 2-Heptadecanone (0.05 mole) in anhydrous diethyl ether was added to a stirred mixture of ethyl acetate (0.1 mole) and sodium hydride (0.05 mole) in anhydrous ether. After completion of the reaction, the crude 2,4-nonadecanedione was recovered following acidification of the reaction mixture. After purification of the product via the copper chelate (17), a 41% yield of 2,4-nonadecanedione, based on 2-heptadecanone, was obtained. The mass spectrum has a parent peak at  $m/e$  296. The calculated molecular weight for  $C_{19}H_{36}O_2$  is 296.4.

### Synthesis of $\Delta^{12}$ -2,4-Heneicosanedione

Oleic acid was converted to oleoyl chloride with oxalyl chloride (18). Oleoyl chloride was allowed to react with ethoxymagnesiummalonic ester. Following acidolysis of the acylmalonic ester (11), the crude  $\Delta^{10}$ -2,4-nonadecanone was purified on a Florisil column followed by

distillation under high vacuum. The product exhibited IR absorption maxima at 2860, 1690, 1440, 1340, and 1160  $\text{cm}^{-1}$ ; this spectrum was similar to that of a reference methyl ketone, 2-heptanone. The  $\Delta^{10}$ -2-nonadecenone was condensed with ethyl acetate in the presence of sodium hydride to form  $\Delta^{12}$ -2,4-heneicosenedione. The latter compound was purified by the formation of its copper chelate with cupric acetate in aqueous ethanol, followed by regeneration of the diketone with dilute acid. The yield was 29.5% from the methyl ketone. A pure sample of the diketone was obtained by preparative GLC on a 3% OV-1 column. The mass spectrum has a parent peak at  $m/e$  322. The calculated molecular weight for  $\text{C}_{21}\text{H}_{38}\text{O}_2$  is 322.3. The IR absorption spectrum exhibited maxima at 2900, 2820, 1680 (weak), 1600, 1350, 1230, 960, 770, and 750  $\text{cm}^{-1}$ .

#### Synthesis of 2,4-Pentacosanedione

Starting with docosanoic acid, which was converted to the acid chloride by means of thionyl chloride, 2,4-pentacosanedione was prepared by the procedure outlined above for 2,4-nonadecanedione. The yield was 47% based on docosanoic acid. The product was purified by preparative GLC on 3% OV-1 column at 240 C. The mass spectrum had a parent peak at  $m/e$  380. The calculated molecular weight for  $\text{C}_{25}\text{H}_{48}\text{O}_2$  is 380.6.

#### Preparation of Methoxime and 2,4-dinitrophenylsemicarbazone Derivatives of 2,4-Diketones

The methoxime derivatives were prepared by heating various 2,4-diketones with an excess of ca. 2.5% methoxyamine hydrochloride in pyridine for 3 hr at 60 C (16). The reaction mixture was diluted with water and the mixture was extracted with petroleum ether. The organic phase was washed with 1N hydrochloric acid, then with saturated aqueous sodium bicarbonate, was dried and evaporated.

The 2,4-dinitrophenylsemicarbazones were prepared as described by Tumlinson et al. (14).

#### Thin Layer Chromatography (TLC) of 2,4-Diketones

On Bakerflex Silica Gel Film IBF with chloroform as the developing solvent, 2,4-diketones with chain lengths from  $\text{C}_5$  to  $\text{C}_{23}$  had  $R_f$  values between 0.4 and 0.5. The compounds were located by their ultraviolet absorption or by their reaction with 2,4-dinitrophenylhydrazine. TLC thus had a potential application only in separation of the 2,4-diketones as a group. Great care had to be exercised to avoid the inadvertent formation of

chelates with di- and trivalent cations which may be present as impurities in some silica gel preparations. Silica Gel G is useless, as it contains calcium sulfate as a binder.

#### Alkaline Hydrolysis of 2,4-Diketones

An 0.1 mg sample of the 2,4-diketone was placed in a 7 ml vial and 0.2 ml 5% aqueous potassium hydroxide was added. The vial was closed with a tightly fitting Vacutainer stopper, and it was heated at 80 C in a heating block overnight. A 1.0 ml sample of the headspace was withdrawn with a 2.5 ml gas-tight syringe and injected onto a Porapak Q column (6 ft x 1/8 in., stainless steel) operated at 130 C (19). Confirmation of the acetone peak was made by headspace analysis of an aqueous solution of acetone which had been treated in the same manner as the sample. The fatty acid was extracted from the aqueous solution in the vial after acidification of the contents and was identified as the methyl ester by GLC on a 10% UCW98 column.

#### Periodate-Permanganate Oxidation of 2,4-Diketones

Samples of 2,4-diketones were oxidized with the von Rudloff periodate-permanganate reagent (20) as described by Chang and Sweeley (21) with the modification that the tert-butanol was omitted. The carboxylic acids resulting from the reaction were identified as their methyl esters by GLC.

## RESULTS AND DISCUSSION

Satisfactory yields of 2,4-diketones were obtained by the method outlined in the previous section. Attempts to condense higher fatty acid esters, such as ethyl palmitate or stearate, with acetone in the presence of sodium hydride were unsuccessful in our hands; free fatty acid was usually isolated instead of the expected diketone. Swamer and Hauser (10), however, have reported the preparation of 2,4-pentadecanedione, as well as other  $\beta$ -diketones, by this latter method. They carried out the reaction in a ball mill, whereas we employed conventional glass apparatus with magnetic stirring.

Gas liquid chromatographic retention time data for 2,4-diketones are presented in Table I. It was convenient to express the retention times of the homologues with respect to 2,4-heptadecanedione. Over the range of chain lengths investigated, the plot of  $\log$  (retention time) vs. number of carbon atoms in the chain was linear for the saturated compounds. On UCW98,  $\Delta^{12}$ -2,4-heneicosenedione eluted just before

TABLE I

## Relative Retention Time Data for 2,4-Diketones

Diketone chain length and type	4% Carbowax on Diatoport S, 6 ft x 1/8 in. SS (He 30 ml/min)		10% UCW98 on Diatoport S, 6 ft x 1/8 in. SS (He 30 ml/min)		
	T 210 C	T 220 C	T 200 C	T 230 C	T 240 C
C <sub>7</sub> :0	0.50		0.029		
C <sub>9</sub> :0	0.89		0.054		
C <sub>10</sub> :0	0.123		0.082		
C <sub>12</sub> :0	0.225		0.166		
C <sub>13</sub> :0		0.311		0.299	0.322
C <sub>14</sub> :0		0.414		0.407	0.430
C <sub>15</sub> :0				0.550	
C <sub>17</sub> :0	1.00 <sup>a</sup>	1.00 <sup>b</sup>	1.00 <sup>c</sup>	1.00 <sup>d</sup>	1.00
C <sub>18</sub> :0				1.36	
C <sub>19</sub> :0		1.80		1.85	1.84
C <sub>20</sub> :0		2.39		2.50	2.36
C <sub>21</sub> :1		3.35		3.08	
C <sub>21</sub> :0		3.18		3.40	3.14
C <sub>23</sub> :0		5.52		6.25	5.59
C <sub>25</sub> :0				11.50	

<sup>a</sup>Retention time, 5.42 min.<sup>b</sup>Retention time, 3.70 min.<sup>c</sup>Retention time, 21.55 min.<sup>d</sup>Retention time, 4.75 min.

TABLE II

## Relative Retention Times of 2,4-Diketone Methoximes and 2,4-Dinitrophenylsemicarbazones

Compound	Relative retention time of methoxime	Relative retention time of 2,4-dinitrophenyl semicarbazone
	Column: 3% OV-17 Temperature: 230 C	Column: 3% OV-1 Temperature: 200 C
2,4-Tridecanedione	0.153	0.130
2,4-Heptadecanedione	0.535	0.493
2,4-Nonadecanedione	1.00 <sup>a</sup>	1.00 <sup>b</sup>
2,4-Heneicosanedione	1.84	2.03
2,4-Tricosanedione	3.43	

<sup>a</sup>Retention time, 3.74 min<sup>b</sup>Retention time, 7.60 min

2,4-heneicosanedione, whereas, on Carbowax 20M, the relative order was reversed, in accordance with their expected behavior. Retention time data for the methoxime and 2,4-dinitrophenylsemicarbazone derivatives of several 2,4-diketones are summarized in Table II. Derivatization with methoxyamine hydrochloride in pyridine has been employed by us in the confirmatory identification of 2,4-diketones obtained by preparative GLC of ketonic material from tissue extracts.

Because of their tautomeric structure, 2,4-diketones undergo silylation with BSA-pyridine or TBT. The trimethylsilyl derivatives may be resolved on columns containing UCW98 stationary phase. These derivatives have not,

however, been employed extensively in our investigations.

Infrared absorption spectra of several 2,4-diketones, obtained with liquid films, showed strong absorption at 2940-2780  $\text{cm}^{-1}$  and at 1580-1600  $\text{cm}^{-1}$ ; this latter absorption band is characteristic of the  $\beta$ -diketone structure (22). In the ultraviolet,  $\beta$ -diketones absorb strongly in the region of 270 nm ( $n \rightarrow \pi^*$  transition). Because of enolization of active hydrogens, the absorbance is much greater than the summation of the contributions of two simple C=O groups (22). Thus, the observed molar absorption coefficient of 2,4-heptadecanedione in 2,2,4-trimethylpentane at 271 nm was 11,000 ( $E_{1\%}^{1\text{cm}} = 408$ ). The absorption maximum at 273.5 nm

TABLE III  
Melting Point Data for 2,4-Diketones

Compound	Melting point °C	Eutectic with Azobenzene °C	Reported melting point	Reference
2,4-Tridecanedione	21.1	—	24-27	5
2,4-Tetradecanedione	26.3	—	28	7
2,4-Pentadecanedione	32.0	—	31-32	7
			31.5-32	9
2,4-Heptadecanedione	42.5	35.0	39	7
2,4-Octadecanedione <sup>a</sup>	46.5	38.5	42	7
2,4-Nonadecanedione <sup>a</sup>	50.5	41.0	49	7
2,4-Eicosanedione <sup>a</sup>	53.7	44.0	51	7
2,4-Heneicosanedione <sup>a</sup>	58.0	46.5	52.5	7
	59			
2,4-Tricosanedione <sup>a</sup>	62.5	49.7	57	7
2,4-Pentacosanedione <sup>a</sup>	66.8	53.7	—	—

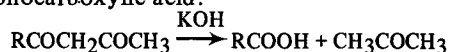
<sup>a</sup>Purified by preparative gas liquid chromatography (GLC) on 3% OV-1.

for 2,4-diketones in 95% ethanol was shifted to 295 nm on the addition of sodium methoxide or sodium hydroxide, with enhanced absorbance at the latter wavelength.

Melting points of several 2,4-diketones, and their eutectic melting points with azobenzene, are summarized in Table III, along with previously reported values. Samples of the diketones were conveniently purified from traces of lower homologues and other impurities by preparative GLC on 3% OV-1.

Mass spectra of 2,4-heptadecanedione, 2,4-nonadecanedione, 2,4-heneicosanedione,  $\Delta^{12}$ -2,4-heneicosanedione, and 2,4-pentacosanedione gave molecular weights (m/e of parent ion peaks) of 268, 296, 324, 322, and 380, in accord with the calculated values. In all of these mass spectra, a prominent peak at m/e 100 occurred; this peak has been attributed to the ion  $C_5H_8O_2^+$ , formed by  $\beta$ -elimination of the long alkyl chain by a McLafferty rearrangement (23).

Periodate-permanganate oxidation of saturated 2,4-diketones yielded the expected monocarboxylic acids containing three carbon atoms less than the diketones.  $\Delta^{12}$ -2,4-Heneicosanedione was cleaved also at the double bond, the final products being nonanedioic and nonanoic acids. The products of alkaline hydrolysis of a 2,4-diketone are acetone and the expected monocarboxylic acid:



The ease with which acetone could be identified by headspace analysis rendered this reaction a valuable confirmatory test for 2,4-diketones when it was applied to preparative GLC fractions from tissue extracts.

The 2,4-diketones are known compounds;

however, they have not hitherto been identified as natural products. Since they occur in biological materials as complex mixtures of homologues, it was necessary to isolate them as a group from tissue lipids, to resolve this fraction into individual components, and to identify the latter by physical and chemical techniques and by comparison with authentic 2,4-diketones, the synthesis and properties of which are described in this communication.

The ability of this group of compounds to form chelates with di- and trivalent metal ions, including  $Ca^{++}$  and  $Mg^{++}$ , is well known. Some of the lower homologues, especially 2,4-pentanedione, and their fluorinated analogues have been employed as analytical reagents because of this property. The metal chelates so formed have the properties of organic compounds; they are soluble in organic solvents such as chloroform and methylene chloride, and some of lower molecular weight may be sublimed. The 2,4-diketones also react readily with compounds possessing a primary amino group to form Schiff bases, and, under appropriate conditions, they may react with -SH as do other active carbonyl compounds. Their widespread distribution in mammalian tissues suggests that they may have some important function or functions in the life process quite apart from their antihistamine activity. Further investigation of these compounds could result in useful therapeutic applications.

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McGill University, for supplying mass spectra of the  $\Delta^{12}$ -C<sub>21</sub> and C<sub>25</sub> compounds. This work was supported by a grant from the Medical Research Council of Canada.

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