Self-Radiolysis of Tritium-labelled Fatty Acid Esters *

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SUMMARY

High specific activity methyl-butyrate-2,3-H^3 and methyl-stearate-9,10-H^3 were stored for approximately three months. The self-radiolysis decomposition products were identified by means of radio-gas chromatographic methods and the isotopic dilution technique. Rather high G-values were found for the parent acids and for the destroyed ester molecules.

INTRODUCTION

The accurate quantitative study of the radiation chemistry of a given organic compound requires a precise determination of both the nature and amount of its radiolytic products and of the small fraction of the compound itself destroyed by the radiation. In order to overcome the considerable analytical difficulties, sensitive techniques have been employed, including IR or UV spectrometry, column, paper and gas chromatography, mass spectrometry, etc. [1].

Radioactive or stable isotopes have also found application [2] but their use has been so far restricted by the limited availability of labelled molecules; in this connection, the use of tritiated molecules shows considerable promises owing to the fact that high specific activity, high purity tritiated molecules can be often obtained with conventional techniques (hydrogenation, hydrolysis, etc.).

Whenever this possibility arises it is not necessary to irradiate the tritiated sample with an external radiation source, since the beta particles from the decay of tritium deliver to the compound an amount of energy which can be easily calculated and produces an appreciable self-radiolysis [3].

The irradiation products can be easily detected, thanks to their activity, at very low concentration, while the amount of the compound decomposed can be precisely determined using reverse dilution methods [4].

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Finally, the activity distribution found in the products of the self-radiolysis, for a compound labelled in known position with ^{3}H, makes it possible to gain valuable information on the mechanism of the radiolysis.

The present paper refers to the identification of the products formed in the self-radiolysis of two fatty acid methyl esters, whose radiolysis had not been investigated so far. These two esters, methyl butyrate-2,3-^{3}H and methyl stearate-9,10-^{3}H, prepared from methyl crotonate and from methyl oleate, respectively, have been chosen in order to study the influence on the radiolytic yields of the following parameters : the number of carbon atoms of the acid, the phase effect and the dose effect.

In addition, a comparison is made between the results of the present experiments and those previously obtained using a sample of methyl stearate-^{3}H, with a higher specific activity, prepared from methyl linoleate [5].

EXPERIMENTAL PART

1. — *Methyl stearate-9,10-^{3}H*

Methyl stearate-9,10-^{3}H was prepared by hydrogenation of methyl oleate with a mixture of hydrogen and tritium (ca. 3 curies) on the Adams platinum oxide, in petroleum ether. The tritiated stearate was crystallized several times from water : ethanol in order to ensure its chemical and radiochemical purity. The specific activity of the purified ester was 314 mC/mmole, as measured by means of an inonization chamber with a standard deviation $\pm 0.5\%$.

2. — *Methyl butyrate-2,3-^{3}H*

Methyl butyrate-2,3-^{3}H was prepared by hydrogenation of methyl crotonate with a mixture of hydrogen and tritium (ca. 3 curies) on the Adams platinum oxide, in petroleum ether. The purification of the tritiated methyl butyrate was carried out by preparative GLC with a 8 mm i.d. 3 m long column, containing polyethylenglicol 400 on Celite C-22, at 105° C, with nitrogen as carrier gas, at flow rate of 2 liters per hour.

The specific activity of the pure ester was 244.8 mC/mmole with a standard deviation $\pm 0.5\%$.

3. — *Storage of the samples*

The tritiated samples were stored within 10 ml Pyrex ampoules, under the conditions stated in Table I. After storage the ampoules containing the radioactive esters were connected to a vacuum system and the gaseous radiolytic products collected by means of a Töpler pump and analysed by GSC on a 4 mm i.d., 2 m long column containing molecular sieves 5 Å, at 100° C. The effluent gases were introduced into a flow ionization chamber, coupled with a Cary mod. 32 vibrating reed electrometer.

The outgassed methyl butyrate and stearate samples were diluted with a known amount of the correspondent inactive esters in the ratio 1 : 50; one

fraction of these diluted samples was employed to detect the radioactive impurities and the remaining was diluted again and purified by GLC. The specific activity of these diluted samples was measured with an ionization chamber according to the Wilzbach technique [6].

4. — Radioactive impurities determination

I. — *Methyl butyrate*-2,3-³H.

a) Low-boiling point esters. — These impurities were detected by means of radio gas chromatography, employing inactive esters as carriers. The GLC column, 2 meters long, 8 mm i.d. was packed with polyethylenglicol 400 on Celite C-22 and operated at 100° C, with carrier gas nitrogen at 2 liters per hour.

b) Free acids. — An aliquot of the stored ester was diluted with inactive methyl butyrate, dissolved in ether and stirred with an aqueous solution of 10 % NaOH. The aqueous layer was acidified, extracted with ether, esterified with diazomethane and analysed under the same conditions described for the low-boiling esters.

c) Fractions boiling between 144 ° and 194 ° C. — One butyrate aliquot was added to o-xilene and decaline. The active products distilling with o-xilene were collected and the total amount of radioactivity measured. The decaline residue contained high boiling tritiated compounds, probably polymers; the radioactivity of the residue was measured.

d) Neutral fractions boiling between 144° and 194° C. — One butyrate aliquot was saponified with 10 % NaOH, extracted with ether; the neutral fraction treated with o-xylene and decalin as in the preceding point *c*).

II. — *Methyl stearate*-9,10-³H.

a) Low-boiling point esters. — The same procedure was used as for the methyl butyrate.

b) Total free acids. Palmitic, heptadecanoic, stearic acid. — Suitable inactive carriers were added to stearate, and the total acid fraction separated by neutralization. The palmatic, heptadecanoic and stearic acids after esterification with diazomethane, were coolected by preparative gas chromatography, using 3 m long, 8 mm i.d. columns, packed with polyethylenglycol succinate on « Celite » C-22, at 205° C.

c) Heptadecane. — The heptadecane present in the stearate was determined adding the inactive hydrocarbon; the sample was then saponified, the neutral fraction extracted in ether, and purified by preparative GLC.

d) Palmitic and heptadecanoic methyl esters. — After adding the inactive carriers, one aliquot of the stearate was purified by preparative gas chromatography, as described in the previous sections.

e) High-boiling point fractions. — The radioactive stearate was added to a known amount of behenic acid, and distilled off at 215° C (15 mm Hg); inactive methyl stearate was then added three times, and distilled off together

with some fractions of the behenic acid (b.p. 260° C, at 15 mm Hg). The residue was weighed and its activity measured.

RESULTS

The absorbed dose has been calculated assuming that all the energy released in tritium decay (average energy $5.6 \cdot 10^3$ eV) was absorbed by the labelled samples.

The *per cent* decomposition has been determined by the reverse dilution analysis method, correcting the results for the tritium decay during the storage time. The G_{-M} values (number of molecules destroyed per 100 eV of energy absorbed by the compound) have been calculated by the percentual decomposition.

The results are summarized in Table I.

The activity present in the gaseous products has been directly evaluated from the radio gas chromatographic peaks, by means of a suitable calibration factor with a standard deviation of 5 %.
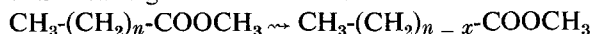
From these data, the G values for the gaseous products shown in Table II have been deduced.

Table III and IV give the activity and the G values for the identified products in both cases. The *per cent* activity found in every identified product, except the gaseous ones, was determined by the reverse dilution analysis, on the fractions purified by GLC.
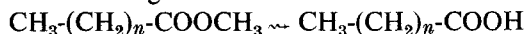
DISCUSSIONS

The scarce literature on the radiation chemistry of organic esters R-COO-R" contains some evidence that the bonds between the oxygen and the two alkyl groups break preferentially in the radiolysis. According to this view three major processes may account for the bulk of radioactive impurities found in the stored tritiated butyrate and stearate :

1° — The breaking of one C-C bond :
$$CH_3\text{-}(CH_2)_n\text{-}COOCH_3 \rightsquigarrow CH_3\text{-}(CH_2)_{n-x}\text{-}COOCH_3$$
2° — The breaking on the ester bond CO-R" :
$$CH_3\text{-}(CH_2)_n\text{-}COOCH_3 \rightsquigarrow CH_3\text{-}(CH_2)_n\text{-}COOH$$
3° — The breaking of the C-OR" bond :
$$CH_3\text{-}(CH_2)_n\text{-}COOCH_3 \rightsquigarrow CH_3\text{-}(CH_2)_n\text{-}H$$

The results of Tables IV and III show that the most of the activity lost during the self-decomposition of the ester samples can be accounted for according to these three main processes.

The hydrogen yields in the butyrate and stearate ($G_{H_2} = 0.5$ and 1.35 respectively) are in good agreement with the G-values of the work of Whitehead *et al.* [7] on carboxylic acids, i.e. the hydrogen molecules/100 eV increase with the number of carbon atoms of the acid. In the case of stearate — where the

TABLE I. Decomposition of the labelled fatty acid esters

Compound	Quantity (mg)	Specific activity mC/mmole	Storing conditions	Time (days)	Absorbed energy		Decomposition %	$G\text{—}M$
					eV/g	Mrad		
Methyl butyrate-2,3-^{3}H	179.6	244.8	1. under vacuum	100	4.30×10^{21}	68.8	27.7	38.00
	179.6		2. in air	100	4.30×10^{21}	68.8	30.6	41.80
Methyl stearate-9,10-^{3}H	314.15	314.0	under vacuum	71	1.37×10^{21}	22.0	24.9	36.55
Methyl stearate-^{3}H (from linolate)	61.08	1289.0	1. under vacuum	190	1.47×10^{22}	235.0	61.2	8.34
	58.66		2. in air	190	1.47×10^{22}	235.0	60.6	8.28

TABLE II. Self-radiolysis gaseous products

Compound	Storing conditions	G_{H_2}	G_{CH_4}	$G_{C_2H_6}$	$G_{C_3H_8}$	Gas activity in μC per mg of starting ester [1]			
						H_2	CH_4	C_2H_6	C_3H_8
Methyl butyrate	1. under vacuum	0.48	0.25	0.04	0.42	1.33	0.40	0.63	7.20
	2. in air	0.70	0.29	0.05	0.49	2.82	1.08	0.73	8.40
Methyl stearate	under vacuum	1.36	0.82	0.03	—	0.69	0.02	~0.004	—
Methyl stearate (from linolate)	1. under vacuum	1.10	0.55	0.05	—	0.80	0.05	0.05	—
	2. in air	0.73	0.49	~0.006	—	0.69	0.05	0.05	—

[1] The standard deviation of the radio-gas-chromatographic analysis is ± 5 %.

hydrocarbon character is more important — the methyl heptanoate and methyl palmitate are formed according to the different probability of breaking the CH_3-CH_2- and -CH_2-CH_2 bonds; that is, in the ratio 1 : 3. In n-alkanes, A. TOPCHIEV [8] has shown that the probability of the rupture of CH_3-CH_2- bond is approximately five times smaller than that for the dissociation of the -CH_3-CH_2- bond.

TABLE III. Total identified self-radiolysis products of Me-butyrate

Identified products in methyl butyrate-2,3-³H	Activity % [a]		G molecules/100 eV	
	under vacuum	in air	under vacuum	in air
H_2	0.056 [b]	0.120 [b]	0.48 [b]	0.70 [b]
CH_4	0.017 [b]	0.046 [b]	0.25 [b]	0.29 [b]
C_2H_6	0.026 [b]	0.031 [b]	0.04 [b]	0.05 [b]
Methyl acetate	0.018	0.019	0.02	0.03
Methyl propionate Methyl i-butyrate	0.027	0.026	0.04	0.04
Methyl i-valerianate	0.650	0.460	0.89	0.63
Methyl valerianate	0.062	0.099	0.08	0.13
Acetic acid	0.005	0.012	0.01	0.02
Propionic acid i-butyric acid	0.022	0.160	0.03	0.22
Butyric acid	11.840	12.100	16.20	16.50
Fraction boiling 144°-194° C	0.790	0.830	1.09	1.13
Fraction boiling 194° C (Polymers ?)	1.460	1.710	2.00	2.34
Total per cent found in products	15.283	15.973	21.55	22.57
Total per cent of decomposition	27.450	30.650	37.55	41.95

[a] All the values are based on the initial methyl butyrate activity.
[b] The G-values and activity % have been calculated on the gas-chromatographic and radio-gas-chromatographic peak; the others by the isotope dilution method.

The yields of total acids and total esters found in the butyric and stearic
ester :

Methyl butyrate-2,3-^{3}H	Methyl stearate-9,10-^{3}H
$G_{acids} = 16.20$	$G_{acids} = 10.77$
$G_{esters} = 1.03$	$G_{acids} = 14.00$

TABLE IV. Total identified self-radiolysis products of Me-Stearate

Identified products in methyl stearate-9,10-^{3}H under vacuum	Activity % [a]	G molecules/100 eV
H_2	0.067 [b]	1.36 [b]
CH_4	0.002 [b]	0.82 [b]
C_2H_6	0.0004 [b]	0.03 [b]
Heptadecane	0.382	0.56
Methyl formiate	0.002	~0.003
Methyl acetate	0.002	~0.003
Methyl propionate Methyl i-butyrate	0.002	~0.003
Methyl butyrate	0.006	~0.009
Methyl palminate	7.220	10.60
Methyl heptadecanoate	2.312	3.40
Stearic acid	4.439	6.52
Palmitic acid	1.065	1.57
Heptadecanoic acid	0.524	0.77
Fraction boiling 260° C at 15 mm Hg (Polymers ?)	3.868	5.68
Total free acids	7.297	10.72
Total per cent of products	21.165	33.18
Total per cent of decomposition	24.900	36.55

[a] All the values are based on the initial methyl stearate activity.
[b] The G-values and activity % have been calculated on the gas-chromatographic and
radio-gas-chromatographic peak; the other ones by the isotope dilution method.

show a different breaking probability of the C-C versus the O-R bonds as a consequence of the different number of carbon atoms. The production of the « parent acid » accounts for the 45 % of the self-decomposition in the case of methyl butyrate, and for only the 15 % in the case of the methyl stearate.

It is interesting that the acid production, in the case of methyl butyrate, is approximately independent of the presence of oxygen. Probably, the reaction mechanism does not involve thermal radicals as intermediates. Similar results have been recently obtained by MAC LACHLAN [9], who irradiated benzyl acetate in cyclohexane : in addition to a high yield of acid, he observed that the presence of oxygen did not affect the *per cent* decomposition of the ester and the yield of the acid.

The self-decomposition for the same compound is strongly dependent — under similar storing conditions — on the total dose absorbed.

A sharp decrease for the G values, has been found for two samples of methyl stearate, going from 22 Mrad to 235 Mrad of total dose absorbed, as shown in Table I.

WAGNER et al. [10] have found in ester radiolysis large (up to sevenfold) differences in most product yields, varying inversely with dosage, in the range of 50 to 500 Mrad. A similar sharp decrease in G_{-M} values has been found by STANFORD and EVANS [11] in storing tritiated compounds, for increasing absorbed doses.

CONCLUSION

In the self-radiolysis of tritiated esters the G values have been found to be markedly affected by the dose absorbed by the sample. This can be explained with the fact that, due to the large conversion of the starting material, its concentration in the sample steadily decreases, while there is a parallel increase of the concentration of the radiolysis products.

Therefore, the amount of starting material destroyed per unit time is not constant, and its concentration in the sample does not decrease linearly with the time.

Under these conditions, it is not correct to calculate the G values over the entire storing period, since systematically different results are to be expected, depending on the total conversion of the sample. This explains, for instance, the wide scatter of the published G_{-M} values of tritiated substances with different specific activity stored for comparable period of time under otherwise similar conditions.

In addition, it should be pointed out that some final products of the radiolysis could act as radical scavangers or excitation acceptors, reducing further the decomposition rate of the stored compound.

In conclusion, the self-radiolysis of tritiated fatty acid-esters has been found to give rise to tritiated products, whose nature and amounts are consistent with the results obtained in the irradiation of inactive esters with external sources.

The relatively high conversion of the samples make it necessary, when expressing the radiolytic yields by means of the G values, to take into account the actual amount of the starting material remaining in the system at any given time.

AKNOWLEDGEMENTS

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