FLAME IONIZATION DETECTOR RESPONSE FOR THE CARBONYL CARBON ATOM IN THE CARBOXYL GROUP OF FATTY ACIDS AND ESTERS

R. G. ACKMAN AND J. C. SIPOS

Fisheries Research Board of Canada, Technological Research Laboratory, Halifax, Nova Scotia (Canada)

(Received February 14th, 1964)

INTRODUCTION

On the basis of observations that carbonyl carbon atoms, including those in a number of esters of aliphatic acids, gave no effective response in flame ionization detectors[1,2], it has been assumed that the carboxyl carbon atoms in the fatty acids themselves may not make an effective contribution to molecular response in this type of gas chromatography detector. Since quantitative analysis of fatty acid mixtures has been difficult it is only recently that this assumption has been questioned by KABOT AND ETTRE[3]. The experimental conditions of these authors involved the use of inert supports to prevent adsorption of acids in packed columns, or of Golay columns.

The present study compares the data of KABOT AND ETTRE[3] with other data (ACKMAN AND BURGHER[4]) obtained by a different technique involving addition of formic acid vapor to the carrier gas to suppress adsorption on columns prepared with conventional supports, and extends the application of the latter technique to some higher acids. The conclusions reached indicate that in the higher fatty acids the carboxyl carbonyl carbon atom may give up to a full carbon atom response on a relative weight basis. A limited study of the molar responses for various esters of fatty acids indicates that the deficiency in response for the lower esters, excepting formates, is 1.5 carbon atoms, and for the higher esters falls to 1 carbon atom. Formate esters of the lower alcohols apparently do not have as great a deficiency in molar response.

EXPERIMENTAL

In acid studies the Wilkens Hi-FY type flame ionization detector, oven, recorder and integrator were those previously described[4]. The column primarily employed was of stainless steel tubing, 6 ft. in length and 1/8 in. O.D., packed with 5 % neopentyl glycol adipate polyester on 60–80 mesh Gas-Pack F (a Teflon impregnated support obtained from Chemical Research Services, Inc., Addison, Ill., U.S.A.). Operating conditions for valeric and higher acids were: column temperature 180°, injection port temperature 250°, carrier gas (helium) at 10 p.s.i., with air and hydrogen constant at normal flow rates for this detector. Analyses of certain lower acids were carried out under similar conditions at a column temperature of 140°. Formic acid vapor was added to the carrier gas in these studies.

Analyses of esters were carried out with the same column at appropriate column temperatures and carrier gas flow rates, and also on a column of stainless steel tubing, 8 ft. in length and $1/8$ in. O.D., packed with 10 % SE-30 silicone polymer on Gas-Pack F support. Other studies were carried out with a Barber-Colman model 10, fitted with a model 5121 flame ionization detector. Columns were of glass, 6 ft. in length and $1/8$ in. I.D., packed with either 15.5 % EGSS-Y organosilicone polyester (Applied Science Laboratories, State College, Pa., U.S.A.) on 100–120 mesh Gas-Chrom P support, or 5 % SE-30 on 70–80 mesh Anakrom ABS support. Appropriate operating conditions for the higher esters were: column temperature 100°, injection port temperature 280°, carrier gas (argon) at 4 to 8 p.s.i. For lower esters and hydrocarbons the respective temperatures were 60° and 120°, with carrier gas at 2 to 4 p.s.i. Air and hydrogen flow rates were constant in all experiments and normal for this detector. A Minneapolis-Honeywell 5 mV recorder fitted with a Disc Instruments, Inc., ball and disc integrator was used with this apparatus.

All injections were performed with Hamilton No. 7001-N microsyringes. Samples for the Wilkens apparatus were normally run as 10 % solutions in carbon disulphide, the syringe being rinsed and partly filled with 0.0003 ml of solvent, then with approximately 0.0003 ml of solution, and the plunger withdrawn a further 0.0001 ml before puncture of the injection septum. This procedure gave better reproducibility than straightforward injection of solutions of sample. Samples for the Barber-Colman apparatus were injected, on occasion, by a similar procedure using carbon disulphide or neohexane as a solvent where appropriate, but in cases of more volatile materials injection of the sample mixture could be effected by normal procedure, since this detector is less susceptible to overload. All analyses carried out on the two machines were in satisfactory agreement. Sample mixtures were in all cases of two materials only, with proportions such that both peaks gave nearly full scale response. No attenuation changes were made during analyses.

The fatty acids employed were prepared in this laboratory, as were the higher esters, with purities exceeding 99%. The hydrocarbons and lower esters employed were reagent grade and gave only one significant peak on gas chromatography.

RESULTS AND DISCUSSION

Acids

The previous data of ACKMAN AND BURGHER[4], and of KABOT AND ETTRE[3] (Table I, literature columns) were converted so that the highest common acid (valeric) was taken as 100 response units, with other acids in proportion for equal weights. These data were plotted in a fashion similar to that of Fig. 1, as relative response against the weight percent "active" carbon atoms. This was initially done on the assumption that the carboxyl group made no contribution to response in the flame ionization detector. The experimental curve started above the theoretical line and then fell below it, indicating that the assumption that the carboxyl group gave no response must be incorrect. Accordingly the data were recalculated on the basis that for the higher fatty acids the carboxyl group might give a full response. In this case, as indicated in Fig. 1, both sets of data approached the theoretical line satisfactorily.

It was considered desirable to establish the minimum chain length at which the deficiency in contribution of the carboxyl group carbonyl carbon atom becomes significant and the molar responses for valeric and caproic acids, relative to pelargonic

TABLE I

AVERAGE RELATIVE RESPONSES FOR EQUAL WEIGHTS OF FATTY ACIDS

Fatty acid	Ackman and Burgher[a]			Kabot and Ettre[a]				
	Literature[a]	Relative to		Literature		Relative to		
		$C_5 = 100$	$C_{10} = 119$			$C_5 = 100$	$C_{12} = 122$	
	(c)	(c)	(c)	(c)	(d)	(c)	(c)	(d)
Acetic (C_2)	47	48	46	48	—	53	52	—
Propionic (C_3)	74	75	73	69	63	77	75	69
Butyric (C_4)	90	91	88	86	85	96	94	93
Isobutyric (C_4)	—	89[b]	84[b]	—	—	—	—	—
Valeric (C_5)	99	100	97	90	92	100	98	100
Valeric (C_5)	—	100[b]	95[b]	—	—	—	—	—
Isovaleric (C_5)	100	101	98	—	—	—	—	—
Caproic (C_6)	105	106	103	—	—	—	—	—
Caproic (C_6)	—	109[b]	103[b]	—	—	—	—	—
Enanthic (C_7)	—	—	—	100	100	111	109	109
Caprylic (C_8)	—	—	—	104	—	116	113	—
Pelargonic (C_9)	—	123[b]	116[b]	—	—	—	—	—
Capric (C_{10})	—	126[b]	119[b]	?	—	?	?	—
Lauric (C_{12})	—	—	—	112	—	125	122	—

a Average of Tween and NPGA results.
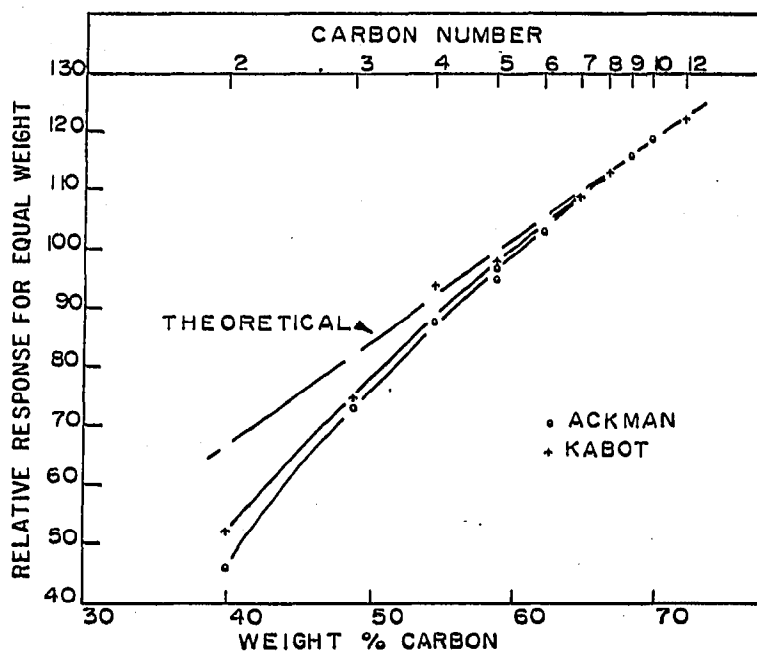b Present study.
c Packed column.
d Golay column.

Fig. 1. Plot of relative response for equal weights of acids against weight percent of all carbon atoms in the molecule.

and capric acids, were established from a large number of results employing the described operating conditions with formic acid vapor in the carrier gas. These data are given in Table II, with the average relative molar responses for valeric and caproic

acids being 472 and 584, respectively, when the higher acids are assigned values based on a response of 100 units per carbon atom. The molar response data were then converted to response for equal weights and correlated with the previous data through caproic acid, the combined data then being expressed in proportion to the highest acid (capric) assuming the latter to give the full theoretical response for all ten carbon atoms. Similarly the data of KABOT AND ETTRE[3] were converted to a full response for lauric acid. The plots of the two sets of results are shown in Fig. 1, where despite some scattered points there is fair agreement between the two sets of data, and both sets of

TABLE II

AVERAGE MOLAR RESPONSES FOR SOME ACIDS RELATIVE TO OTHER ACIDS
AS DETERMINED IN THE PRESENT STUDY

Fatty acid	Molar response relative to		
	Caproic = 584	Pelargonic = 900	Capric = 1000
Isobutyric (C$_4$)	359	—	—
Valeric (C$_5$)	—	475	469
Caproic (C$_6$)	—	583	586

data indicate as suggested by the results of these authors that within probable experimental error the relative weight response of fatty acids is theoretical for the full number of carbon atoms in the chain, including the carboxyl group carbonyl carbon atom, for fatty acids with more than six or seven carbon atoms.

A previous suggestion[5] that for these higher chain lengths equal weights of acids give nearly equal response is only a fair approximation since the presence of two oxygen atoms gives a more substantial rate of change in relative weight percent "active" carbon with increments of methylene groups in the case of the acids than in hydrocarbons of corresponding chain lengths[6]. The same considerations apply to analyses of esters of aliphatic acids[7] (cf. ref. 8). Correction factors[3,6,7] are therefore necessary for precise results even with the higher acids and their esters.

On a molar response basis the deficiency in response for the carboxyl carbonyl atom for valeric acid indicates that the example selected for comment by KABOT AND ETTRE[3], the relative responses of valeric, pelargonic and capric acids, cannot give an integral multiple for either of the higher acids (Table II). In view of other differences in instrumentation it is unlikely that the small differences in the results are due to the presence of formic acid vapor in the carrier gas, although an apparent case of suppression of ionization has been discussed elsewhere[9]. The reason for the deficiency in carbonyl carbon atom response in the lower fatty acids is not apparent, since there is no evidence for an alternating value such as might be associated with an inductive effect from the hydrocarbon chain. Although previous reports[10] (cf. ref. 5) suggested that isovaleric and isobutyric acids might have markedly higher relative responses than the corresponding normal acids, the results obtained in the present study and elsewhere suggest that iso-acids give substantially similar results. In the present comparison of the relative responses of isobutyric acid with that of normal butyric acid from a previous study it should be noted that a similar bias is shown by the normal valeric acids from the two sets of data, suggesting that a slight systematic error accounts for the lower relative response given for isobutyric acid (Table II). This

view is in agreement with results for normal butanol and isobutanol, and for normal butyl acetate and isobutyl acetate[5] (*cf.* ref. 1), where both forms have substantially the same response. This is an indication that further investigation of the "cracking" of these molecules in the flame of the detector is required. KABOT AND ETTRE[3] have suggested that the polar functional group affects the response of the adjacent methylene carbon atom, an effect neutralized by the lengthening hydrocarbon chain in the higher acids.

It must be emphasized that these findings in regard to acids refer to the *relative* responses *among* the various acids. The molar or weight responses in relation to other materials such as hydrocarbons, as pointed out for acids by KABOT AND ETTRE[3], may be quite different, depending on the different fundamental polyatomic groups characteristic of each class of organic homologues (*cf.* refs. 1 and 2).

Methyl esters

A few ester analyses of materials analogous in chain length with the higher acids studied were initially run on the Wilkens apparatus with the polyester column in order to verify that no systematic error was involved in the latter analyses. The expected integral molar responses (for one carbon atom = 100 response units), based on the number of "active" carbon atoms as the total less the carboxyl group, as indicated by PERKINS *et al.*[2], could not be obtained for pairs of esters such as methyl caproate and methyl caprate (Table III). These anomalous responses were found to be

TABLE III

AVERAGE RELATIVE MOLAR RESPONSE OF ESTERS RELATIVE TO OTHER ESTERS

Compound investigated	Reference material	Reference molar response	Compound molar response	Carbon atom deficiency	Column
Methyl valerate	Methyl pelargonate	900	450	1.5	Pol
Methyl valerate	Methyl caprate	1000	446	1.5	Sil
Ethyl valerate	Methyl pelargonate	900	561	1.4	Pol
Methyl caproate	Methyl pelargonate	900	560	1.4	Pol
Methyl caproate	Methyl pelargonate	900	572	1.3	Sil
Methyl caproate	Methyl caprate	1000	558	1.4	Pol

independent of operating variables, injection procedure, and the presence or absence of formic acid vapor in the carrier gas. Similar results were obtained with the Barber-Colman apparatus.

Accordingly a variety of esters were investigated to obtain responses relative to two hydrocarbons. To extend the range of analyses both polyester and silicone columns were employed in the two different types of apparatus. This also permitted varying the order of appearance of components in hydrocarbon–ester mixtures. The results are given in Table IV.

The assumption of integral (100 units per carbon atom) reference relative responses for heptane and decane (Table IV) does not seriously affect comparison of data in relation to the two hydrocarbons, since the differences in weight percent "active" carbon for these materials is small. This difference may be somewhat larger in the case of methyl pelargonate and methyl caprate (Table III) and is probably

associated with the generally slightly higher responses for the lower esters relative to methyl pelargonate.

The data in Tables III and IV indicate that in methyl and ethyl esters of the shorter chain acids, excepting formic, the net deficiency in carbon atom response is normally 1.4 to 1.5 carbon atoms. This declines as the fatty acid chain lengths exceed 8 carbon atoms to a deficiency of 1 carbon atom for acids with 10 or more carbon atoms.

TABLE IV

AVERAGE RELATIVE MOLAR RESPONSES OF ESTERS RELATIVE TO HYDROCARBONS

Compound investigated	Reference material	Reference molar response	Compound molar response	Carbon atom deficiency	Column
Heptane	Decane	1000	703	—	Pol
Heptane	Decane	1000	694	—	Sil
Methyl formate	Heptane	700	87	1.1	Sil
Ethyl formate	Heptane	700	179	1.2	Sil
Propyl formate	Heptane	700	284	1.2	Sil
Methyl acetate	Heptane	700	157	1.5	Sil
Ethyl acetate	Heptane	700	252	1.5	Sil
Methyl propionate	Heptane	700	249	1.5	Sil
Propyl propionate	Heptane	700	463	1.4	Sil
Methyl butyrate	Heptane	700	355	1.4	Pol
Ethyl valerate	Heptane	700	550	1.5	Pol
Ethyl valerate	Heptane	700	549	1.5	Sil
Methyl caproate	Decane	1000	563	1.4	Pol
Methyl caprylate	Decane	1000	779	1.2	Sil
Methyl pelargonate	Decane	1000	898	1.0	Sil
Methyl caprate	Decane	1000	995	1.0	Sil

These results could be interpreted to mean that the alcohol portion of the molecule is split off to give an alcohol with a response deficiency of 0.5 carbon atom[2,6], and that in the lower acids the carboxyl carbonyl carbon atom also has a deficiency of 1 carbon atom. In the esters of higher acids either a different scission of the ester linkage may take place, giving a hydrocarbon residue in lieu of the alcohol, or the carboxyl group carbon atom begins to have a significant response parallelling that for the higher fatty acids.

The formate esters appear to be somewhat different in character, although this has not been investigated for those esters containing longer chain alcohols. The formates examined by PERKINS et al.[2] appeared consistent excepting for methyl formate. The lower value for this ester in relation to the two other formate esters examined (Table IV) may therefore be a property of this unique ester. The intermediate net carbon atom deficiency for the formate esters suggests that in the cracking of the molecules a proportion of hydrocarbons are formed from and in addition to the alcohols, since formic acid itself gives no response[4,11]. Thus the molar response results for methyl, ethyl and propyl formates, 87, 179 and 284, respectively, may be compared on the same basis to the results reported for the corresponding alcohols[1,5], 75, 170 and 256, respectively. The comparison seems valid since the result for ethyl acetate in the present study is 252 as against 253 from the same literature source. It should, however, be noted that these relative responses for methanol and ethanol are somewhat higher than suggested by PERKINS et al.[2,6].

The comparative responses for these esters, relative to hydrocarbons, were obtained by a different procedure from that of PERKINS et al.[2]. The latter authors used an open tube and separate injections of materials with calibrated syringes. The binary molar response results for ethyl, propyl and isobutyl acetates obtained by STERNBERG et al.[1] by another procedure, are respectively 253, 375 and 476, relative to heptane as 700 units per mole, and are in moderate agreement with the present results. Differences in the relative volatility of the materials injected did not seriously affect the present results, since similar relative response values were obtained with or without the addition of a more volatile solvent such as carbon disulphide. Sample sizes were varied in each analysis to check for detector overload which might occur with the first peak. The absence of this effect was also demonstrated by the similarity of results when peak order was reversed in certain analyses by changing from polyester to silicone columns. For methyl formate and methyl acetate, relative to heptane, smaller samples did give greater response for the ester relative to heptane. The peak height ratios did not change in such analyses and the greater response for these two esters was observed to be due to a relatively greater tailing of the ester peak. This suggests that a fixed amount of the ester sample may be temporarily adsorbed on the support or in the rubber connectors of the Barber-Colman columns. At high attenuations this tailing material would not be recorded or integrated.

Polycarboxylic acids were not investigated, but KUKSIS AND VISHWAKARMA[12] have observed in column analyses that the responses for some of the Krebs cycle acid esters, possessing in some cases hydroxyl and carbonyl functional groups in addition to more than one carboxyl group, were apparently proportional to the *total* number of carbon atoms present when compared with octadecane.

SUMMARY

The response of the carboxyl group in free fatty acids, although markedly less than the equivalent response for a methylene carbon atom in the lower fatty acids, approaches this value in acids with six or more carbon atoms. The relative responses for these higher fatty acids are then proportional to the relative weight percent carbon content on a weight basis.

In esters a complex molar response pattern is evident, apparently due to scission of the ester linkage with the lower acids to give an alcohol with a response of half a methylene carbon atom, and an acid or carboxyl group fragment with little or no response. In the methyl esters of saturated fatty acids with nine or more carbon atoms the net loss in response falls to the equivalent of one methylene carbon atom and the relative response is then proportional to the relative weight percent carbon content based on the number of carbon atoms in the fatty acid chain. Formate esters apparently give slightly higher responses than other esters of the same net number of carbon atoms. This suggests that the differences observed in the various esters may be due in the cases of those esters with higher responses to formation of a hydrocarbon in lieu of an alcohol during the initial scission of the ester group linkages. Alternatively in the esters other than formates the carboxyl group may give an increasing response as the fatty acid chain length increases.

REFERENCES

1 J. C. STERNBERG, W. S. GALLAWAY AND D. T. L. JONES, in N. BRENNER, J. E. CALLEN AND M. D. WEISS (Editors), *Gas Chromatography, Proc. 3rd Intern. Symp.*, Academic Press, New York, 1962, p. 231–267.
2 G. PERKINS, JR., G. M. ROUAYHEB, L. D. LIVELY AND W. C. HAMILTON, in N. BRENNER, J. E. CALLEN AND M. D. WEISS (Editors), *Gas Chromatography, Proc. 3rd Intern. Symp.*, Academic Press, New York, p. 269–285.
3 F. J. KABOT AND L. S. ETTRE, *J. Gas Chromatog.*, 1, No. 10 (1963) 7.
4 R. G. ACKMAN AND R. D. BURGHER, *Anal. Chem.*, 35 (1963) 647.
5 L. S. ETTRE, *J. Chromatog.*, 8 (1962) 525.
6 G. PERKINS, JR., R. E. LARAMY AND L. D. LIVELY, *Anal. Chem.*, 35 (1963) 360.
7 R. G. ACKMAN AND J. C. SIPOS, *J. Am. Oil Chemists' Soc.*, 41 (1964) 377.
8 L. S. ETTRE AND F. J. KABOT, *J. Chromatog.*, 11 (1963) 114.
9 R. G. ACKMAN AND J. C. SIPOS, *J. Chromatog.*, 13 (1964) 337.
10 E. EMERY AND W. E. KOERNER, *Anal. Chem.*, 33 (1961) 146.
11 J. E. LOVELOCK, *Anal. Chem.*, 33 (1961) 162.
12 A. KUKSIS AND P. VISHWAKARMA, *Can. J. Biochem. Physiol.*, 41 (1963) 2353.