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Note

Effect of derivative structure on flame-ionization detector response of amino acid oxazolidinones

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A simple procedure for the conversion of all protein amino acids into cyclic derivatives, suitable for determination by gas chromatography, has been introduced in previous studies[1–4]. The exact derivatization conditions were determined for amino acids possessing the same or similar reactive groups and the particular mixtures, mostly amino acids in homologous series, were analysed with the use of a flame-ionization detector (FID)[1,4].

In order to evaluate the influence of the derivative structure on the response behaviour, the approach used by Islam and Darbre[5] for the trifluoroacetylated (TFA) amino acid methyl esters was used in this work. However, the absolute molar responses in coulombs per mole were determined directly from the known values for the attenuation ($4 \cdot 10^{-10}$ A for 250 mm full-scale deflection of the integrator) and the chart paper speed (10 mm/min), *i.e.*, a hypothetical area of 250 mm^2 corresponds to $4 \cdot 10^{-10}$ A $\cdot 6$ sec $= 2.4 \cdot 10^{-9}$ C. When compared with the peak area of an exact amount of the derivatized compound injected (*n*-hexadecane was used as the internal standard), the absolute molar responses in coulombs per mole were obtained (Table I). By plotting the found responses against the carbon number of the derivatives in the homologous series, it was possible to determine by extrapolation the responses for compounds with higher carbon numbers that were not recorded by the FID (Fig. 1). The difference between a hypothetical response, being proportional to the actual number of carbon atoms in the molecule, and the lower response found, indicates the presence of specific groups or atoms joined to a carbon atom that prevent it from being recorded as a methyl group.

As the response of hydrocarbons in the FID is generally accepted as being maximal and directly proportional to the number of carbon atoms in the molecule, the dashed line with *n*-hexadecane passes through the origin and the found value of its molar response for C_{16} (3.60 C/mol) agrees with the previously published value[5].

The regression line for the homologous series of the oxazolidinones of amino acids with (A) a carbon-linked chain and also (B_1) a thio-ether bond cuts the abscissa at a "non-effective" value of 2.4. Because of the identical lines for S-alkylated amino acids (Nos. 12–14 in Table I) and amino acids with a carbon-linked side-chain (Nos. 1–11), it is obvious that the thio-ether bond does not reduce the responses of the adjacent carbon atoms, *i.e.*, the compound behaves in the FID like the same compound without the sulphur atom. The same was found by Islam and Darbre[5]

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TABLE I

MOLAR RESPONSES OF DERIVATIZED AMINO ACIDS (N,O-HFB AND N,O-TFA OXAZOLIDIDONES AND *n*-HEXADECANE IN A FLAME-IONIZATION DETECTOR

No.	*Compound*	*Number of carbon atoms**	*Molar response (coulomb/mole)*
—	*n*-Hexadecane	16	3.60
	(A) Amino acids with carbon-linked chain		
1	Glycine (Gly)	5	0.59
2	Alanine (Ala)	6	0.82
3	α-Aminobutyric acid (Aba)	7	1.04
4	α-Aminoisobutyric acid (Aiba)	7	1.08
5	Norvaline (Nval)	8	1.26
6	Valine (Val)	8	1.28
7	Norleucine (Nleu)	9	1.48
8	Leucine (Leu)	9	1.51
9	Isoleucine (Ile)	9	1.53
10	α-Aminocaprylic acid (Aca)	11	1.93
11	Phenylalanine (Phe)	12	2.16
	(B) Amino acids with alkyl substituent on polar group		
12	S-Methylcysteine (Cysm)	7	1.05
13	Methionine (Met)	8	1.26
14	Ethionine (Eth)	9	1.46
15	Sarkosine (Sar)	6	0.69
16	Proline (Pro)	8	1.11
17	Pipecolic acid (Pipa)	9	1.42
18	Tryptophan (Trp)	14	2.34
	(C) Diaminodicarboxylic acids		
19	Diaminosuccinic acid (Dasca)	10	0.98
20	Diaminopimelic acid (Dapa)	13	1.62
21	Diaminosuberic acid (Dasba)	14	1.84
22	Lanthionine (Lan)	12	1.43
23	Cystine (Cys)	12	1.25
24	Homocystine (Hcys)	14	1.51
	(D) Diaminomonocarboxylic acids		
25	Diaminobutyric acid (Daba)	(a) 9	0.91
		(b) 11	1.32
26	Ornithine (Orn)	(a) 10	1.10
		(b) 12	1.55
27	Lysine (Lys)	(a) 11	1.30
		(b) 13	1.75
	(E) Hydroxyamino acids		
28	Serine (Ser)	(a) 8	0.80
		(b) 10	1.25
29	Threonine (Thr)	(a) 9	1.02
		(b) 11	1.47
30	Tyrosine (Tyr)	(a) 14	1.94
		(b) 16	2.33
31	α-Methyltyrosine (α-CH_3-Tyr)	(a) 15	2.13
		(b) 17	2.50
32	Hydroxyproline (Hyp)	(a) 10	1.14
		(b) 12	1.48
	(F) Other amino acids		
33	Histidine (His)	13	1.58
34	Arginine (Arg)	(a) 13	1.53
		(b) 17	1.79
35	Homoarginine (Harg)	(a) 14	1.84
		(b) 18	2.10

* (a) Acylation with TFAA; (b) acylation with HFBA.

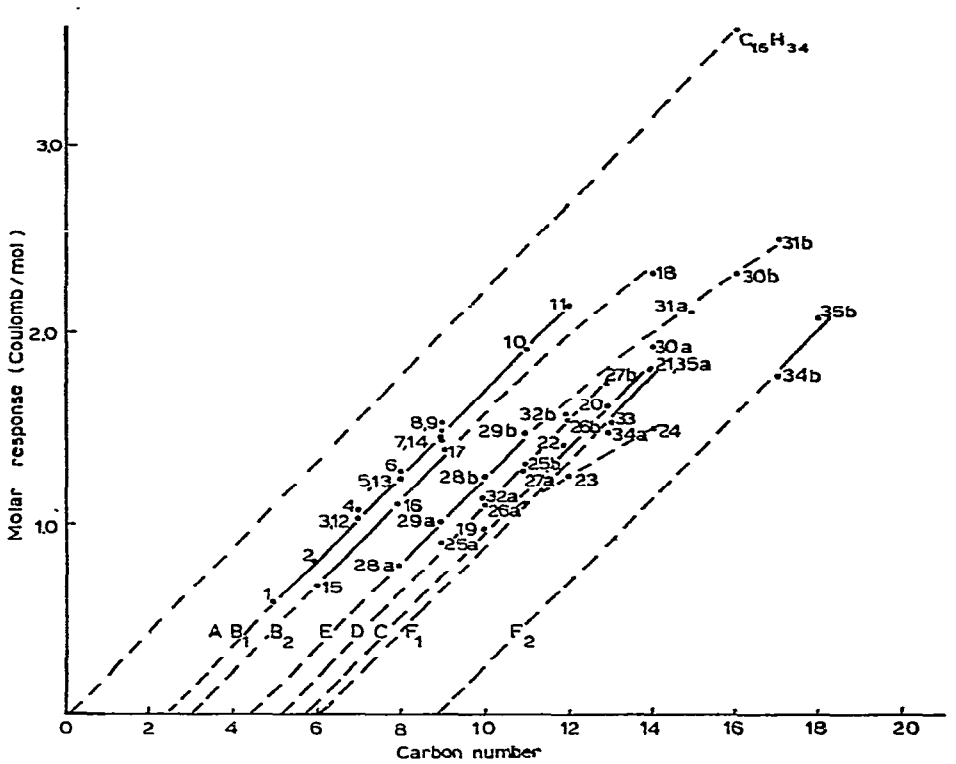
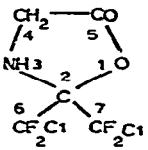


Fig. 1. Relationship between FID molar responses and number of carbon atoms in derivatized compounds. On extrapolation the regression lines cut the abscissa at a point that gives the value of the non-effective carbon number. For identification of compounds, see Table I.

with the N-TFA methyl esters of amino acids, where the responses for Met and Nval were nearly identical, as they were in our work. In contrast, the reason for a lower value for Met as occurred for example, with the N,O-heptafluorobutyryl (HFB) alkyl esters[6–8], probably lies in the derivatization procedure used.

After subtracting the corresponding "non-effective" value of 2.4, a response value of 2.6 will be obtained for the oxazolidinone ring, *i.e.*, glycine after condensation. According to some earlier studies, summarized by Islam and Darbre[5], a carbon atom in the neighbourhood of an oxygen atom (ester bond or carbonyl group), *i.e.*, the second and the fifth carbon atoms of the ring, has a zero response. Therefore, the fourth carbon atom and both chlorodifluoromethyl groups are responsible for the response of the ring.



As the N-containing groups also lower the response of an adjacent carbon atom, *e.g.*, to a value of 0.25–0.75 of its full response, as was found by Sternberg

et al. (ref. 9, p. 231), or to a value of about 0.3–0.4 for imino acids in our work (see further), it follows that the two perhalogenated methyl groups have a full or nearly full response (if we assign them the value of 2.0, there still remains a value of 0.2–0.3 for the carbon atoms in the ring). Identically, as the molar responses of O-TFA and O-HFB esters of Ser and Thr oxazolidinones (regression line in Fig. 1) are close to that of Ala and Aba and to Nval and Nleu in the latter instance, it follows that the terminal groups $-CH_2-O-CO-CF_3$ and $-CH_2-O-CO-C_3F_7$ have the same response as 1 and 3 carbon atoms, respectively. Provided that the two oxygen atoms hinder the adjacent carbon atoms to be recorded, the trifluoromethyl and, as mentioned above, also the chlorodifluoromethyl groups seem to have responses identical with those of their aliphatic equivalents. However, this strange phenomenon (the fluoroalkanes have been repeatedly confirmed to yield a lower detector response than alkanes[10]) is nowadays not exceptional, as discussed later. A possible explanation in our work is that owing to the presence of oxygen in the neighbourhood of a perhalogenated carbon chain, the negative effect of the halogen presence on the response may be reduced.

The hydroxylated aromatic amino acids (Tyr, α-CH_3-Tyr) afford, contrary to expectation, approximately a one carbon atom lower response (curving of the regression line E at the top), which is probably caused by incomplete esterification of the phenolic group (yield about 90%) under the specified reaction conditions[4]. However, it is interesting that a similar decline in the response of the N-TFA methyl ester of Tyr was found[5] even when a breakdown of the derivative in the column was said to be responsible for the partial losses.

The N-TFA and N-HFB acylated oxazolidinones of diaminocarboxylic acids (regression line D) also show a one carbon atom lower response in comparison with the O-TFA and O-HFB acylated forms with an equal number of carbon atoms. Regression line D cuts the abscissa at a value of 5.1. After subtracting the non-effective carbon number for the oxazolidinone ring (2.4), this leaves 2.7 for the $-CH_2-NH-CO-CF_3$ group and 4.7 for the $-CH_2-NH-CO-C_3F_7$ group as the unrecorded carbon portions, *i.e.*, only 0.3 and 2.3 carbon atoms in the latter instance are recorded. As the secondary amino group causes a carbon number reduction of 0.6, as follows for the imino acids (Nos. 15–17) by comparison of the regression lines A (B_1) and B_2, or perhaps of 0.7 in this instance, the recorded value of 0.3 belongs to the methylene group, whereas the $-CO-CF_3$ moeity, connected to a carbon linkage by a nitrogen bond, has a zero response. Considering some previous work on amino acid derivatization[6,11] equally differing values of molar responses of the N,N-diacylated amino acids in comparison with the N,O-diacylated forms can be found. These findings support the preceding tentative interpretation that a response of a halogenated alkyl (carbonyl) chains seems to be influenced by the heterogeneous atom in the neighbourhood.

The diaminodicarboxylic acids (regression line C) have a lower response than twice the response of one oxazolidinone ring, *i.e.*, the response of Dasca (4.3) is not equal to twice the response of Gly (5.2). This means that the response of the methylene group in the oxazolidinone ring is reduced by connection of the antipole. The response of Lan corresponds to a response of a hypothetical diaminoadipic acid (not commercially available), which supports the fact that the thioether bond does not lower the responses of adjacent carbon atoms. The decreased values for Cys

(—10%) and Hcys (—20%) are not caused by the disulphide bond present, but they indicate partial absorption of the derivatives in the column packing used[4]. The same applies to Trp (with a decrease in response of about 5%).

The molar response of the N^{im}-IBOC oxazolidinone of His fits the values of Arg and Harg TFA-oxazolidinones, finding themselves on regression line F_1 (the HFB derivatives form regression line F_2), and this is the only reason for putting His on this line with a non-effective carbon number of 6.0. Considering the structrue of the derivatized His[4], the response should, in fact, be approximately one carbon atom higher, and this also follows from the sharp elution curve (ref. 4, Fig. 8) for this compound. Even under the best analytical conditions about 10% of His is absorbed by the packing. The N,N-diacylated guanidino group in the molecules of Arg and Harg oxazolidinones was expected to have, according to the preceding results, a response identical with that of the acylated terminal amino group in the molecules of Orn and Lys oxazolidinones. However, N,N-diacylated guanidine actually has a higher response with the TFA groups (effective carbon number equal to 2.1) and a lower one with the HFB groups (3.3). We cannot give any explanation for this phenomenon, but a similar response of Arg can also be found with the other derivatized forms[6,11].

In accordance with the results of Islam and Darbre[5], we noted with the oxazolidinones the same response behaviour as that of amino acid isomers. The isomers show a higher response with an increase in branching of the carbon chain in the order Ile > Leu > Nleu, Val > Nval, Aiba > Aba. However, as was shown with hydrocarbon compounds (ref. 9, p. 307), the differences are small and statistically not significant.

In conclusion, the found values of the molar responses can be helpful in reconsidering several previous erratic representations of response–structure relationships[6–8] and they seem to confirm some recent studies[12–14] dealing with the unexpectedly high responses of some halogenated compounds in the FID. Thus, the high carbon number fluorocarbons showed response values similar to those of the corresponding hydrocarbons and their relative response values were almost independent of the operating conditions, provided that higher hydrogen flow-rates than usual for hydrocarbons were employed[12]. In another instance, the low carbon number 2-chloro-2-bromo-1,1,1-trifluoroethane was found to have almost the same response as ethane under the usual FID conditions[13]. Other workers have found that by varying the flame composition in favour of a hydrogen-rich flame and with oxygen as the combustion supporter, a completely different response behaviour of halogenated hydrocarbons is to be expected[10,14]. All of these findings indicate that one must be very careful in generalizing the behaviour of the FID towards halogenated compounds.

REFERENCES

1 P. Hušek and V. Felt, *J. Chormatogr.*, 152 (1978) 363.
2 P. Hušek and V. Felt, *J. Chromatogr.*, 152 (1978) 546.
3 P. Hušek, *J. Chromatogr.*, 172 (1979) 468.
4 P. Hušek, V. Felt and M. Matucha, *J. Chromatogr.*, 180 (1979) 53.
5 A. Islam and A. Darbre, *J. Chromatogr.*, 71 (1972) 223.
6 M. A. Kirkman, *J. Chromatogr.*, 97 (1974) 175.

7 P. Felker and R. S. Bandurski, *Anal. Biochem.*, 67 (1975) 245.
8 P. Felker, *J. Chromatogr.*, 153 (1978) 259.
9 N. Brenner, J. E. Callen and M. D. Weiss (Editors), *Gas Chromatography*, Academic Press, New York, 1962, pp. 231 and 307.
10 M. A. Pringuer, J. Porter, T. A. Gough and C. F. Simpson, *J. Chromatogr. Sci.*, 17 (1979) 387.
11 S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 171 (1979) 195.
12 E. Proksch, P. Gehringer and W. Szinovatz, *J. Chromatogr. Sci.*, 17 (1979) 568.
13 J. Gelbičová-Růžičková, J. Novák and J. Janák, *J. Chromatogr.*, 64 (1972) 15.
14 A. E. Karagözler, C. F. Simpson, T. A. Gough and M. A. Pringuer, *J. Chromatogr.*, 158 (1978) 139.