Tetrahedron Vol. 34, pp. 2763–2766
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ANALYSIS OF AMINO ACIDS AS *N*-DITHIOCARBAMIC DERIVATIVES. A COMPARISON OF ELECTRON IMPACT AND FIELD IONIZATION MASS SPECTRA

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(*Received in the UK 21 December 1977; Accepted for publication 3 February 1978*)

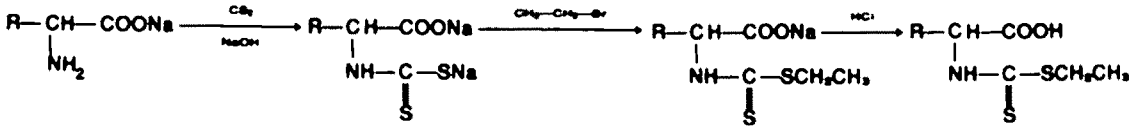
Abstract—Amino acids were converted to their *N*-dithio-alkylcarbamate derivatives by reaction with carbon bisulfide in basic solution followed by reaction with ethyl bromide to form an ethyl thioester. The derivatives are sufficiently volatile for mass spectrometric analysis via the direct inlet probe, without the need for further esterification of the carboxylic acid group. The electron impact spectra exhibit relatively intense molecular ion peaks and structurally diagnostic fragment ions. The general features of the electron impact and field ionization mass spectra are compared.

The vapor phase analysis of free amino acids is severely hampered because of their zwitterionic character. Although it has been possible to obtain interpretable mass spectra of free amino acids, often with well defined molecular ion peaks, by introducing them into the ion source via the direct inlet probe,[1] their generally low volatility and thermal instability renders this procedure impractical. Consequently, chemical derivatization of most amino acids is highly desirable for mass spectrometric analysis using the direct inlet probe method and certainly essential for analysis by combined gas chromatography/mass spectrometry (GC/MS).[2] Derivatization of amino acids involves removal of the polarity associated with the amino and/or the carboxylic function. Typically it is based on the formation of their alkyl ester-*N*-acyl derivatives, of which the *N*-trifluoroacetyl-butyl esters are among the most popular.[3] In addition to *N*-acylation a variety of other functionalities have been considered for derivatization of the amino group in amino acids. Some examples are the *N*-trimethylsilyl,[4] *N*-pivalyl[5] and *N*-dimethylaminomethylene[6] derivatives. Determination of the *N*-terminal amino acid in a peptide chain by Edman degradation involves reaction with phenylisothiocyanate to form its corresponding phenylthiohydantoin. Several variations of this reaction have been explored such as the substitution of methyl- for phenylisothiocyanate[7] or the use of NH_4CNS.[8] In an effort to form a derivative of relatively lower molecular weight. Halpern *et al.*,[9] evaluated the reaction of primary amino groups in esterified amino acids with CS_2. The resulting isothiocyanate methyl ester derivatives exhibited good gas chromatographic properties and structurally informative mass spectra. The simplicity of the carbon bisulfide reaction has, in fact, prompted an examination of its overall utility for the gas chromatographic separation of primary from secondary amines[10] and for the GC/MS analysis of biogenic amines, amphetamines and other biologically active primary amines.[11–13] Secondary amines were shown to form thiocarbamic acids which could then be analyzed by GC/MS as their methyl ester derivatives.[14]

For investigations by mass spectroscopy it is desirable that the chemical derivatives exhibit intense molecular ion peaks and fragmentations characteristic of the molecular structure of the parent compound. In view of the fact that the derivatives resulting from reaction of amino groups with CS_2 have been noted for their generally informative mass spectral patterns, we have conducted a detailed study of the mass spectra of several simple amino acids following derivatization with CS_2. Whereas in previous cases the derivatization procedure involved reaction with CS_2 either with the esterified amino acid[9] or the free amine in an organic solvent[10,11] we have sought to establish the conditions for the direct reaction of the amino acid salts with CS_2. The volatility of the resulting *N*-dithiocarbamic acid derivatives is illustrated from the fact that it was possible to vaporize them into the ion source via the direct inlet probe without even resorting to esterification of the carboxylic group. Judging from the fact that the mass spectra obtained are structurally informative, it is expected that GC/MS analysis of their corresponding methyl esters should be possible with equal effectiveness. We present here the mass spectra of the *N*-dichiocarbamic derivatives of four common amino acids with emphasis on the comparison of their electron impact and field ionization spectra.

EXPERIMENTAL

Derivative formation. The *N*-dithiocarbamic acid derivatives were prepared according to the sequence of reactions outlined in Scheme 1.[15] The detailed procedure for synthesis was as follows: the amino acid (10 mM) dissolved in 50% aqueous ethyl alcohol (25 ml) was treated with 2 N NaOH (10 ml) and 10 mM CS_2, and



Scheme 1.

the mixture stirred at room temperature for 2 h. Following addition of ethyl bromide (10 mM) the stirring was continued for 2 more hours. The solution was then acidified to pH 4 and ethanol was evaporated under vacuum. The residue was extracted three times with ethylacetate and the ester layer was washed with water and dried with anhydrous magnesium sulfate. Following removal of the solvent under vacuum the residue was crystalized as indicated on Table 1.

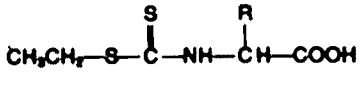
Table 1.

Amino acid derivative	Yield %	Nitrogen analysis Calc %	Found %	Melting point °C	Crystalized from
1. Gly-OH CS_2Et	87	7.83	8.00	120–122	EtOH/ alcohol/ water
2. DL-Ala-OH CS_2Et	83	7.25	7.35	110–112	CCl_4
3. DL-BuOH CS_2Et	91	6.76	6.91	97–99	AcOEt/Petrol ether
4. L-Val-OH CS_2Et	89	6.33	6.42	95–96	AcOEt/Petrol ether

Mass spectrometry. Mass spectra were recorded with a Varian MAT 711 high resolution mass spectrometer. The ion source temp. was 260°C, the ionizing voltage 70 eV, trap current 800 μA and ion accelerating voltage of 8 kV. The derivatives were vaporized into the ion source at a direct inlet probe temp. of 40°C. Field ionization mass spectra were recorded with a combined field desorption-field ionization-electron impact source with an accelerating voltage of 8 kV and an additional potential of +3 kV. The emitter was a 10 μm tungsten wire activated in a Varian apparatus. Precise mass measurements were performed with the peak matching technique using perfluorokerosene as an internal standard. Peak matching was also employed for determination of the exact mass of the ion of *m/e* 62 in the field ionization mass spectra using aceton as the internal standard at a resolving power of M/ΔM of 10,000. Metastable ions falling in the first field-free region of the mass spectrometer were recorded by the metastable defocussing technique.[16]

RESULTS AND DISCUSSION

The compounds considered in this study were the *N*-dithiocarbamic derivatives of glycine 1, DL-alanine 2, DL-α-aminobutyric acid 3 and L-valine 4.



1. R = H 3. R = CH_2CH_3
2. R = CH_3 4. R = $CH(CH_3)_2$

Electron impact mass spectra

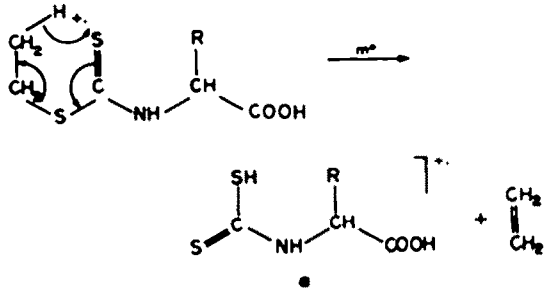
The principal ions in the electron impact mass spectra of 1–4 are summarized in Table 2. The complete electron impact mass spectrum of the *N*-dithiocarbamic derivative of valine 4 is illustrated in Fig. 1 as a representative example of the spectra in the series. In general these derivatives exhibit relatively intense molecular ion peaks, ranging in intensity from 25% to 65% of the base peak. The spectra are dominated by sulfur-containing fragment ions which are common to all four derivatives and whose identity was confirmed by high resolution mass spectrometry. The most significant among them are the following:

Table 2. Partial electron impact mass spectra of compounds 1–4

$M^{+\cdot}$	1 179[b](66)[c]	2 193(28)	3 207(43)	4 221(67)
$[M-C_2H_4]^{+\cdot}$	151(46)	165(14)	179(18)	193(24)
$[M-C_2H_4-HS]^{+}$	118(13)[d]	132(8)[d]	146(12)[d]	160(14)[d]
$[M-C_2H_4-H_2S]^{+\cdot}$	117(77)	131(40)	145(60)	159(55)
$[M-C_2H_4-H_2S-COOH]^{+}$	72(100)	86(100)	100(100)	114(48)
m/e 101				(59)
$CH_3CH_2SH^{+\cdot}$	62(61)	62(47)	62(57)	62(100)

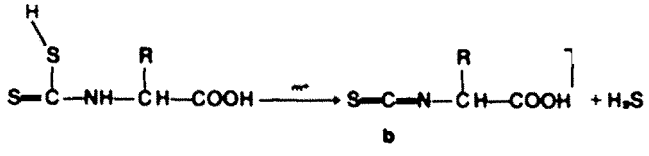
[a]Indicates type of ion. [b]Indicates *m/e* value. [c]Figures in parenthesis refer to relative intensity of indicated ion peaks. [d]Relative intensities have been corrected for isotopic contributions.

[M-28]$^+$. This ion a is produced by elimination of C_2H_4 via a McLafferty rearrangement as shown in Scheme 2.[17]



Scheme 2.

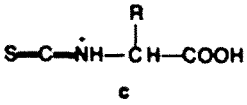
Fig. 1. Electron impact mass spectrum of *N*-dithiocarbamic derivative of L-valine.



b

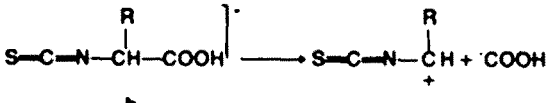
Scheme 3.

[M-28-34]$^+$. The elimination of ethylene is followed by loss of H_2S to yield ion **b** (Scheme 3). Abstraction of one of the labile hydrogens is supported from examination of the spectra of the corresponding N–D, COO–D derivatives. In a manner typical of many sulfur-containing compounds,[18] loss of an SH radical is also observed, yielding an ion for which a structure corresponding to **c** can be written.



c

[M-C_2H_4-H_2S-COOH]$^+$, **d**. Metastable data support the formation of this ion from fragment **b** by direct cleavage beta to the amino group (Scheme 4). It is interesting that no ion of significance corresponding to [M-COOH]$^+$ was observed in the spectra of the four derivatives examined.

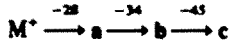


b d

Scheme 4.

Ion **d** yields the base peak in the spectra of derivatives 1, 2 and 3. Its reduced relative abundance in the spectrum of the valine derivative can be explained by the competitive fragmentation processes shown in Scheme 5.

In summary, all of the above fragment ions—except for *m/e* 117 of the valine derivative—contain the characteristic parts of the amine acids, i.e. the portion of the structures by which the amino acids can be distinguished. Thus, identification of the fragmentation sequence:

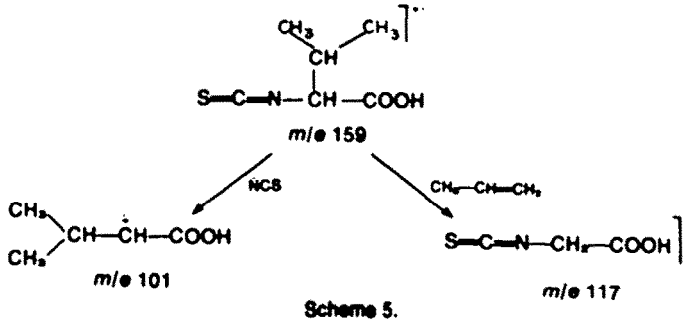


is very important for the structure elucidation of unknown amino acids.

Finally, common to the mass spectra of all four derivatives is the ion of *m/e* 62 e. On the basis of its elemental composition (C_2H_6S) and its 1 amu shift in the spectra of the N–D, COOD derivatives it was identified the ethyl thiol ion ($CH_3CH_2SH^+$, **e**)

Field ionization spectra

Field ionization mass spectra are expected to differ from electron impact spectra, even when the latter are obtained at low ionizing energies. Typically they exhibit intense molecular ion peaks and little fragmentation and



Scheme 5.

Fig. 2. Field ionization mass spectrum of *N*-dithiocarbamic derivative of L-valine.

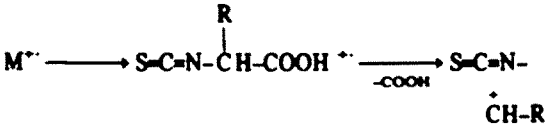
are, in part, characteristic of the chemical processes on the surface of emitters. In the particular case of the *N*-dithiocarbamic derivatives, the field ionization spectra—obtained using aceton as the focusing medium—have base peaks corresponding to the respective molecular ions, with the exception of the alanine derivative. In addition, under the ionization conditions employed—i.e. wire emitters with whiskers activated with benzonitrile—these spectra also exhibit a number of intense fragment ions. The field ionization spectra of the four derivatives are summarized in Table 3. The complete spectrum of the valine derivative 4 is given in Fig. 2.

Table 3. Field ionization mass spectra of compounds 1–4

	1	2	3	4
$M^{+\cdot}$[a]	179[b](100)[c]	193(60)	207(100)	221(100)
$[M-C_2H_4-H_2S]^+$	117(34)	131(100)	145(81)	159(46)
m/e 106				(13)
m/e 100			(6)	
m/e 89		(10)		
m/e 87				(24)
m/e 86		(13)		
$CH_3CH_2SH^+$	62(48)	62(65)	62(34)	62(28)

[a]Indicates type of ion. [b]Refers to *m/e* value. [c]Figures in parenthesis refer to relative intensities of indicated peaks.

Common to all compounds are the ions b and e, formed via two competitive unimolecular fragmentation processes. Beckey's *R* parameters[19,20] where, $R = (I^{i}_{m}/I^{b}_{m})/(I^{i}_{81}/I^{b}_{81})$, are useful criteria for dividing the field ionization fragment ions into two groups. In the first group are ions b and e with *R* parameters of 0.66/2.69 and 0.28/1.37 respectively which are consistent with the one-step fragmentation processes leading to formation of these ions. In the second group are the ions of structure $S{=}C{=}\overset{+}{N}{-}\dot{C}H{-}R$ present in the alanine and α-amino-butyric acid derivatives with an *R* value below 0.1 suggesting a multi-step process:



Acknowledgement—Financial support to Paul Vouros by the National Institutes of Health (GM22787) is gratefully acknowledged.

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