Mass Spectra of Trimethylsilyl Derivatives of some Amino-acids and Peptides

By K. M. BAKER, M. A. SHAW, and DUDLEY H. WILLIAMS*
(*University Chemical Laboratory, Cambridge* CB2 1EW)

Summary Mass spectra of trimethylsilyl derivatives of some amino-acids and di- and tri-peptides contain very easily recognisable M^+ and $M^+ - 15$ peaks, thus making these derivatives useful for molecular-weight determinations on very small amounts of unusual amino-acids and small peptides, or in the analysis of mixtures of such compounds.

ALTHOUGH trimethylsilylation has been used to increase the volatility of numerous compounds prior to examination by mass spectrometry,[1] this technique has not been applied to peptides, where in earlier work almost exclusively acylation of the *N*-terminal end and esterification of the *C*-terminal end was employed,[2] and most recently *N*-permethylation has been utilised.[3] The mass spectra of trimethylsilyl derivatives of some amino-acids have been determined,[4] but no details are available. We have prepared trimethylsilyl derivatives of representative amino-acids and peptides and now report useful aspects of their mass spectra.

The amino-acids tyrosine (as the ethyl ester), lysine, ornithine, and thyronine afforded the derivatives (1)—(4), typically on warming the precursors with bistrimethylsilylacetamide (*ca.* 10-fold excess) and trimethylsilyl chloride (*ca.* 5-fold excess) just below the reflux temperature (*ca.* 80°) until a homogeneous solution was obtained (10—30 min.).[5] Peptides Gly-Gly, Gly-Ala, Gly-Leu, Gly-Val, Gly-Lys, Ala-Gly, Ala-Val, Ala-Leu, and Gly-Met-Gly similarly afforded the derivatives (5)—(13). Mass spectra of (1)—(13) indicated the derivatives to be homogeneous, except that from Gly-Lys, which was a mixture of tris-, tetrakis- [see (9)], and pentakis-trimethylsilyl derivatives, and that from Gly-Met-Gly which gave tris- plus some tetrakis-trimethylsilyl [see (13)] derivatives. Thyronine and Gly-Tyr-glycinamide did not afford the expected tris- and pentakis-trimethylsilyl derivatives but bis- and tetrakis-trimethylsilyl derivatives, respectively [(4) and (14 less one Me_3Si group)].

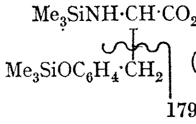
After the subtraction of peaks of no structural utility, and of artefact peaks in some of the spectra, {*m/e* 59 $[CH_3CONH_2]^{+\cdot}$, 73 $[Si(CH_3)_3]^+$, 75 $[(CH_3)_2SiOH]^+$, 147 $[(CH_3)_3SiO{=}Si(CH_3)_2]^+$, 149, 217, 279}, extremely simple mass spectra remained, exhibiting (i) *a very easily recognisable* molecular ion and $M^+ - 15$ pair of peaks and (ii) except for the thyronine derivative (4), an abundant ion at *m/e* 174 (?). In 12 ev spectra, M^+, $M^+ - 15$ and *m/e* 174 were frequently the only ions of significant abundance.

Derivatives containing tyrosine [(1) and (14 less one

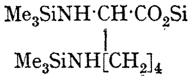
Some common fragment ions (low abundance) observed in the high-mass region of the trimethylsilyl derivatives (1)—(14)

Fragment ion	Origin	Fragment ion	Origin
$M^+ - 72$	$M^+ - (Me_3Si - H)$	$M^+ - 104$	$M^+ - CH_3 - Me_3SiO$
$M^+ - 73$	$M^+ - Me_3Si$	$M^+ - 105$	$M^+ - CH_3 - HOSiMe_3$
$M^+ - 87$	$M^+ - CH_3 - (Me_3Si - H)$	$M^+ - 116$	$M^+ - (CO_2SiMe_3 - H)$
$M^+ - 89$	$M^+ - OSiMe_3$	$M^+ - 117$	$M^+ - CO_2SiMe_3$
$M^+ - 90$	$M^+ - HOSiMe_3$	$M^+ - 133$	$M^+ - CH_3 - HOSiMe_3 - 28$
		$M^+ - 162$	$M^+ - SiMe_3 - OSiMe_3$

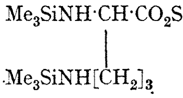
Me_3Si group)] also gave characteristic abundant ions at *m/e* 179 (formally $Me_3SiOC_6H_4CH_2^+$). Analogously, the thyronine bistrimethylsilyl derivative (4) gave abundant ions at *m/e* 271 ($Me_3SiOC_6H_4OC_6H_4CH_2^+$) and *m/e* 199 ($HOC_6H_4OC_6H_4CH_2^+$). The derivatives (2) and (3) from lysine and ornithine gave abundant ions of structural utility due to *a* and *b*, respectively.[6]


(1)

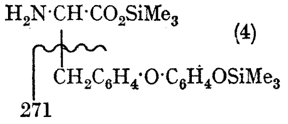
$Me_3SiNH{\cdot}\underset{Me_3SiOC_6H_4{\cdot}CH_2}{CH}{\cdot}CO_2Et$ 179


(2)

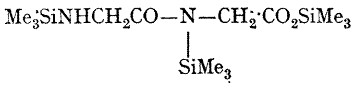
$Me_3SiNH{\cdot}\underset{Me_3SiNH[CH_2]_4}{CH}{\cdot}CO_2SiMe_3$


(3)

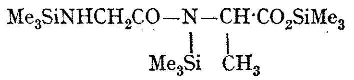
$Me_3SiNH{\cdot}\underset{Me_3SiNH[CH_2]_3}{CH}{\cdot}CO_2SiMe_3$


(4)

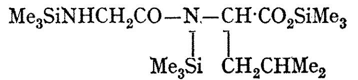
$H_2N{\cdot}\underset{CH_2C_6H_4{\cdot}O{\cdot}C_6H_4OSiMe_3}{CH}{\cdot}CO_2SiMe_3$ 271


(5)

$Me_3SiNHCH_2CO{-}\underset{SiMe_3}{N}{-}CH_2{\cdot}CO_2SiMe_3$


(6)

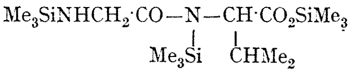
$Me_3SiNHCH_2CO{-}\underset{Me_3Si}{N}{-}\underset{CH_3}{CH}{\cdot}CO_2SiMe_3$


(7)

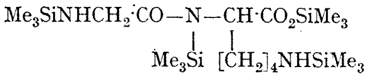
$Me_3SiNHCH_2CO{-}\underset{Me_3Si}{N}{-}\underset{CH_2CHMe_2}{CH}{\cdot}CO_2SiMe_3$

Otherwise, the amino-acid and dipeptide derivatives underwent remarkably few fragmentation reactions. In the high-mass region, *low abundance* fragment ions due to the losses indicated in the Table were common (although some of the losses were observed in one or two of the spectra only).†

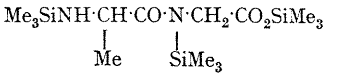
Thus trimethylsilyl derivatives of small peptides do not appear to be suitable for sequencing work, but should be very useful in determining the molecular weight of unusual amino-acids and small peptides available in sub-milligram quantities, and in analysis of mixtures of the same. It is emphasised that the derivatives are obtained in one step and are available for mass spectrometry (requiring only a few μg. of sample) directly after the evaporation of the excess of reagent.


(8)

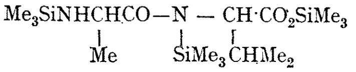
$Me_3SiNHCH_2{\cdot}CO{-}\underset{Me_3Si}{N}{-}\underset{CHMe_2}{CH}{\cdot}CO_2SiMe_3$


(9)

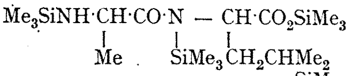
$Me_3SiNHCH_2{\cdot}CO{-}\underset{Me_3Si}{N}{-}\underset{[CH_2]_4NHSiMe_3}{CH}{\cdot}CO_2SiMe_3$


(10)

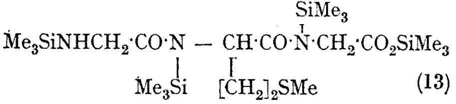
$Me_3SiNH{\cdot}\underset{Me}{CH}{\cdot}CO{\cdot}\underset{SiMe_3}{N}{\cdot}CH_2{\cdot}CO_2SiMe_3$


(11)

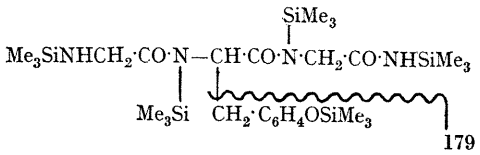
$Me_3SiNH\underset{Me}{CH}CO{-}\underset{SiMe_3}{N}{-}\underset{CHMe_2}{CH}{\cdot}CO_2SiMe_3$


(12)

$Me_3SiNH{\cdot}\underset{Me}{CH}{\cdot}CO{\cdot}\underset{SiMe_3}{N}{-}\underset{CH_2CHMe_2}{CH}{\cdot}CO_2SiMe_3$

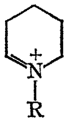

(13)

$Me_3SiNHCH_2{\cdot}CO{\cdot}\underset{Me_3Si}{N}{-}\underset{[CH_2]_2SMe}{CH}{\cdot}CO{\cdot}\overset{SiMe_3}{N}{\cdot}CH_2{\cdot}CO_2SiMe_3$


(14)

$Me_3SiNHCH_2{\cdot}CO{\cdot}\underset{Me_3Si}{N}{-}\underset{CH_2{\cdot}C_6H_4OSiMe_3}{CH}{\cdot}CO{\cdot}\overset{SiMe_3}{N}{\cdot}CH_2{\cdot}CO{\cdot}NHSiMe_3$ 179

This work was supported by fellowships from Imperial Chemical Industries, Limited, (to K.M.B.) and the S. R. C. (to M.A.S.).


a, R=Me_3Si, *m/e* 156
R=H, *m/e* 84


b, R=Me_3Si, *m/e* 142
R=H, *m/e* 70

(*Received, July* 10*th*, 1969; *Com.* 1015.)

---

† The tripeptide derivatives (13) and (14) underwent some additional cleavage reactions which are under investigation.

[1] See, *e.g.*, J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Kreuger, and R. N. Stilwell, *J. Amer. Chem. Soc.*, 1968, **90**, 4182; D. F. Hunt, C. E. Hignite, and K. Biemann, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 378.

[2] For a review, see J. H. Jones, *Quart. Rev.*, 1968, **22**, 302.

[3] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Amer. Chem. Soc.*, 1969, **91**, 3096, and references cited therein.

[4] R. M. Teeter, paper presented as the ASTM Committee E-14 Mass Spectrometry Conference, New Orleans, La., 1962.

[5] For an alternative procedure for the preparation of trimethylsilyl derivatives of amino-acids see J. F. Klebe, H. H. Finkbeiner, and D. M. White, *J. Amer. Chem. Soc.*, 1966, **88**, 3390.

[6] The formation of *m/e* 84 has also been observed from lysine ethyl ester; K. Biemann, J. Seibl, and F. Gapp, *J. Amer. Chem. Soc.*, 1961, **83**, 3795.