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THE USE OF CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR IDENTIFICATION OF RADIATION-INDUCED DNA BASE DAMAGE AND DNA BASE–AMINO ACID CROSS-LINKS

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SUMMARY

Application of capillary gas chromatography-mass spectrometry (GC–MS) to isolation and identification of radiation-induced DNA base damage including DNA base–amino acid crosslinks is reported. γ-Irradiated samples of thymine (thy), thymidine (dT), thymidine-5′-monophosphate (pdT), cytosine(cyt),2′-deoxycytidine (dC), 2′-deoxycytidine-5′-monophosphate (pdC), and mixtures of thy, dT and pdT with tyrosine were used as model systems. Trimethylsilylation was used as the derivatization method. Samples containing nucleosides and nucleotides were first subjected to hydrolysis with formic acid or hydrochloric acid to remove sugar or sugar-phosphate moieties, then trimethylsilylated and analyzed by GC–MS. Trimethylsilyl derivatives of radiation-induced monomeric and dimeric products of the model systems mentioned above were shown to have excellent GC properties and easily interpretable mass spectra. The presence of the molecular ion ($M^{+\cdot}$) and a characteristic $(M-CH_3)^+$ ion in the mass spectra facilitated structural elucidation. The complementary use of *tert.*-butyldimethylsilyl derivatives was also demonstrated. These derivatives provided an intense characteristic $(M-57)^+$ ion in their mass spectra, which may be used to corroborate the molecular weight and to monitor the corresponding compounds in a complex mixture by means of selected-ion monitoring. All gas chromatograms and mass spectra obtained are discussed in detail.

INTRODUCTION

It is generally accepted that DNA is the principal target for ionizing radiation-induced cell killing.[1] Ionizing radiation modifies the heterocyclic bases of DNA, creates base-free sites and causes single and double strand breaks[2]. Another type of DNA damage caused by ionizing radiation is the crosslinking between DNA and proteins and also intra- and inter-molecular crosslinking of DNA[3,4].

The chemical alterations induced by ionizing radiation can disturb the biological functions of DNA such as transcription and replication[5]. The formation of DNA-protein crosslinks[6] may also interfere with these functions. However, lesions produced by ionizing radiation in the DNA of a living cell are subject to repair

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processes[7]. Unless rapaired, such damages may be of importance in cell killing, mutagenesis or carcinogenesis[1]. Therefore, elucidation of the chemical nature of radiation-induced modifications in DNA is valuable for an understanding of the biological consequences and repairability of these lesions.

In the past, the effects of ionizing radiation on DNA and its constituents have been extensively studied under various conditions. Chromatography, nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) were the most successful analytical techniques used for isolation, identification and quantitation of radiation-induced products of DNA and its constituents. For instance, thin-layer chromatography, NMR and MS were extensively applied for identification of radiation-induced products of DNA bases[8], and capillary gas chromatography (GC) combined with MS (GC–MS) was successfully used for the analysis of radiation-induced products of the DNA sugar moiety[9–14]. In another instance, high-performance liquid chromatography (HPLC) was used for separation and identification of *cis*-thymine glycol in γ-irradiated cellular DNA[15], and HPLC was also shown to be useful for separation of radiation-induced products of thymine itself[16].

GC with capillary columns is one of the most widely used analytical techniques for separating complex mixtures[17–19]. This technique, especially when combined with MS, provides a highly sensitive and powerful methodology for separation and identification of a variety of organic compounds. In recent years, GC–MS, using mostly conventional packed columns, has played an important role in the characterization of synthetic and natural nucleic acid bases and nucleosides[20–22]; applications of capillary columns have also been reported[23]. Because of low volatility, derivatization is necessary to make the bases and nucleosides suitable for GC–MS analysis. For this purpose, trimethylsilylation appears to be the most useful, since it can be easily carried out and gives trimethylsilyl (TMS) derivatives of bases and nucleosides with excellent GC properties and easily interpretable mass spectra[20,21,24,25].

This paper reviews some recent results from our laboratory on the application of capillary GC–MS to isolation and identification of radiation-induced DNA base damage including DNA base–amino acid crosslinks. Moreover, mass spectra not published previously are also presented and discussed. Trimethylsilylation was used as the derivatization method throughout those studies. In addition, the complementary use of *tert.*-butyldimethylsilyl (t-BDMS) derivatives is demonstrated in this paper. γ-Irradiated samples of thymine (thy), thymidine (dT), thymidine-5′-monophosphate (pdT), cytosine (cyt), 2′-deoxycytidine (dC), 2′-deocycytidine-5′-monophosphate (pdC) and mixtures of thy, dT and pdT with tyrosine (Tyr) were used as model systems.

EXPERIMENTAL*

Materials

Thymine (thy), thymidine (dT), thymidine-5′-monophosphate (pdT), cytosine (cyt), 2′-deoxycytidine (dC), 2′-deoxycytidine-5′-monophosphate (pdC), tyrosine

* Certain commercial equipment, instruments, or materials are identified in this paper in order to specify adequately the experimental procedures. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified are necessarily the best available for the purpose.

(Tyr), and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Sigma (St. Louis, MO, U.S.A.). Acetonitrile and pyridine were obtained from Pierce (Rockford, IL, U.S.A.). N-Methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide was from Regis (Morton Grove, IL, U.S.A.).

Methods

γ-Irradiations. Aqueous solutions of thy, dT, pdT, cyt, dC, pdC, (all 1 m*M*) and of mixtures of thy, dT and pdT with Tyr (each 0.5 m*M* in the mixture) were saturated with oxygen-free N_2O and irradiated in a ^{60}Co-γ-source (up to 440 Gray; dose rate 110 Gray per min). The samples were then lyophilized.

Hydrolysis with formic acid or hydrochloric acid. Irradiated samples of dT, pdT, dC, and pdC were hydrolyzed with formic acid (88%) at 150°C for 30 min in evacuated and sealed tubes. Irradiated mixtures of dT and pdT with Tyr were hydrolyzed with 1 *N* hydrochloric acid at 100°C for 4 h in the same manner. After hydrolysis, the samples were dried in a desiccator *in vacuo* prior to trimethylsilylation.

Derivatization. 2 mg of each sample were trimethylsilylated in PTFE-capped Hypo-vials (Pierce) with 0.2 ml of a mixture of BSTFA and acetonitrile (1:1) by heating for 30 min at 140°C. *tert.*-Butyldimethylsilyl (t-BDMS) derivatives were prepared according to Crain and McCloskey[26].

Gas chromatography. A Hewlett-Packard (Rockville, MD, U.S.A.) Model 5880A microprocessor-controlled gas chromatograph equipped with a flame ionization detector was used. The injection port and detector were maintained at 250°C. Separations were carried out on a fused-silica capillary column (12 m × 0.2 mm I.D.) coated with SE-54 (cross-linked 5% phenyl methylsilicone; wall coated open tubular; siloxane deactivated; film thickness, 0.11 μm) (Hewlett-Packard). The measured efficiency of the column (*n*) was *ca.* 5400 theoretical plates per meter based on the pentadecane peak at 120°C (capacity factor, 6.42; linear velocity of the carrier gas, 32.8 cm/sec). Helium was used as the carrier gas. The glass liner in the injection port was packed with silanized glass wool purchased from Alltech (Deerfield, IL, U.S.A.).

Gas chromatography–mass spectrometry. Mass spectra were taken at 70 eV using a Hewlett-Packard Model 5970A mass selective detector interfaced to the above gas chromatograph. GC conditions were as above. Temperature of the ion source was *ca.* 200°C.

RESULTS AND DISCUSSION

GC–MS of γ-irradiated thymine, thymidine and thymidine-5'-monophosphate

Fig. 1 shows a gas chromatogram obtained from irradiated thy after trimethylsilylation. Peak 1 represents the TMS derivative of thy. Peaks 2–9 correspond to the radiation-induced monomeric products of thy reported previously[8]. Mass spectrum taken from peak 2 is given in Fig. 2. From this mass spectrum, peak 2 is assigned as the TMS derivative of 5,6-dihydrothymine. The molecular ion ($M^{+\cdot}$) at *m/z* 272, $(M - H)^+$ at *m/z* 271 and $(M - CH_3)^+$ at *m/z* 257 were observed; loss of hydrogen or methyl radical from molecular ions of TMS derivatives of bases constitute their major fragmentation pathways[20]. The ion at *m/z* 201 was probably due to methyl ketene elimination from *m/z* 257 as described for the TMS derivative of

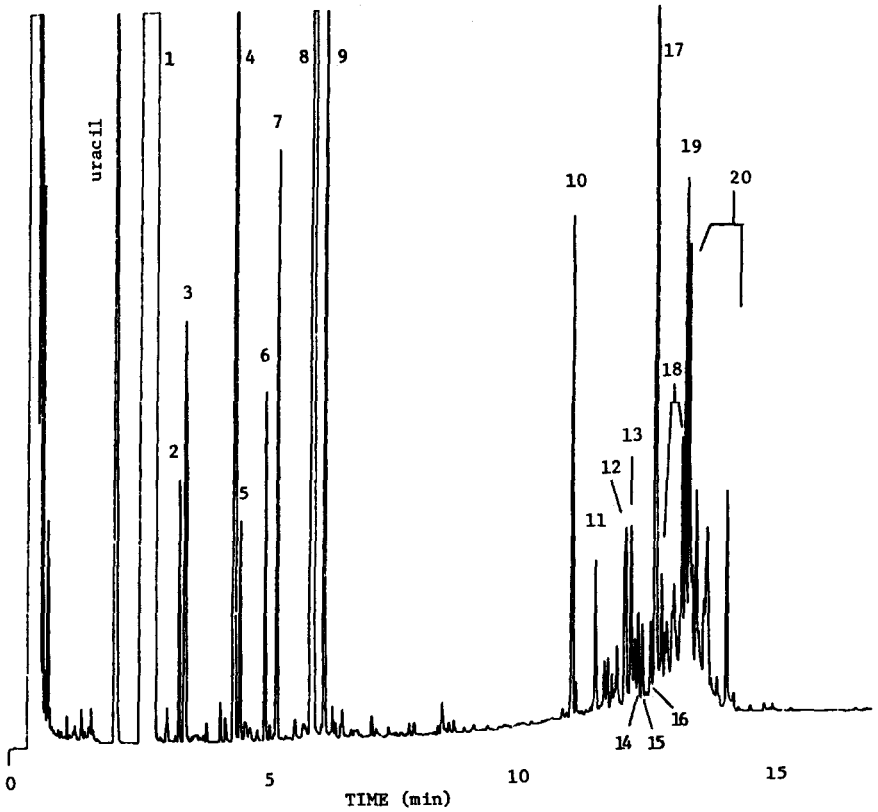

Fig. 1. Gas chromatogram obtained from a γ-irradiated sample of thymine after trimethylsilylation. Column, fused-silica capillary coated with SE-54 (12 m × 0.2 mm I.D.), programmed at 10°C/min from 100 to 250°C. Column head pressure, 100 kPa. Split ratio, 20:1. Uracil was added to the sample as an internal standard before derivatization.

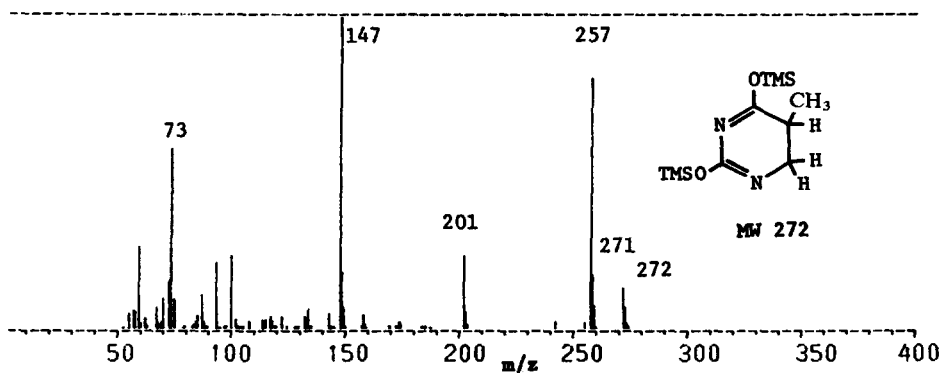

Fig. 2. Mass spectrum taken from peak 2 in Fig. 1.

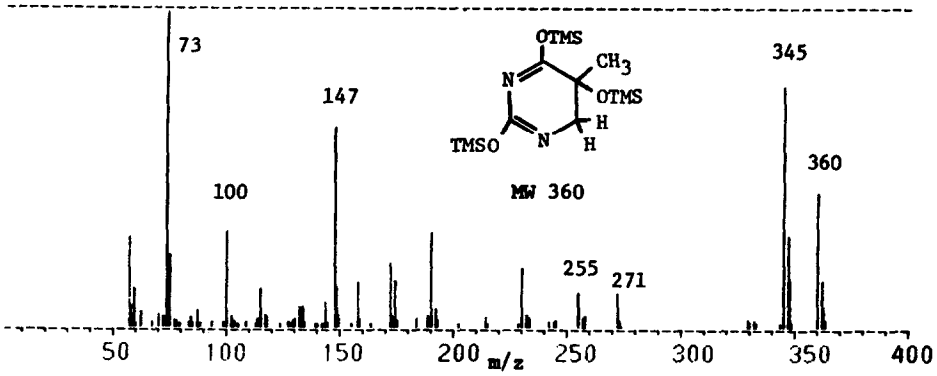

Fig. 3. Mass spectrum taken from peaks 4, 5 and 6 in Fig. 1*.

5,6-dihydrouracil[20]. Ions at *m/z* 73, 75, and 147 are common products of TMS derivative fragmentation and serve no diagnostic purpose. Ion at *m/z* 100 is a characteristic fragment for TMS derivatives of thymine and related compounds[20]. Mass spectrum taken from peak 3 could not be interpreted with certainty, but this peak probably represents the TMS derivative of 5-hydroxy-5-methyl-hydantoin.

Essentially identical mass spectra were obtained from peaks 4, 5 and 6 (Fig. 3). These spectra were assigned to TMS derivative of 5- or 6-hydroxy-5,6-dihydrothymine (see insert in Fig. 3). Abundant $M^{+\cdot}$ and $(M-15)^+$ at *m/z* 360 and 345, respectively, were observed. Loss of OTMS (89 a.m.u.) from $M^{+\cdot}$ probably accounted for the ion at *m/z* 271. *m/z* 255 was due to elimination of trimethylsilanol (HOTMS; 90 a.m.u.) from $(M-15)^+$.

Peak 7 represents TMS derivative of 5-hydroxymethyluracil as confirmed by comparison with authentic material. $M^{+\cdot}$ appeared as the base peak at *m/z* 358 (Fig. 4) and an intense $(M-15)^+$ at *m/z* 343 was also observed. Essentially identical spectra obtained from peaks 8 and 9 (Fig. 5) were attributed to TMS derivatives of *cis* and *trans* 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol). $M^{+\cdot}$ and $(M-15)^+$ were found at *m/z* 448 and 433, respectively. An intense ion was observed at *m/z* 259,

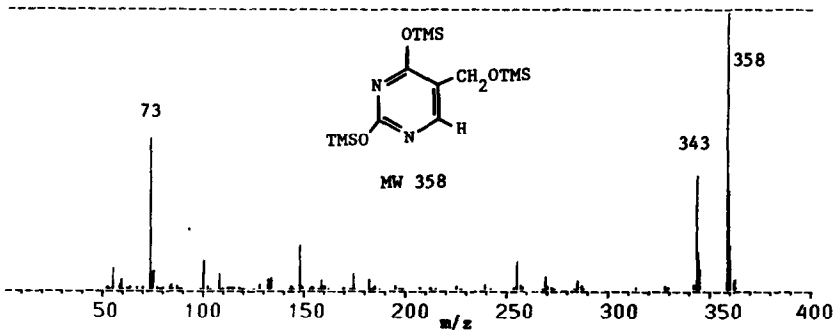

Fig. 4. Mass spectrum taken from peak 7 in Fig. 1.

* Mass spectra of the isomeric compounds shown in this paper were taken from the most intense GC peaks representing these isomeric compounds.

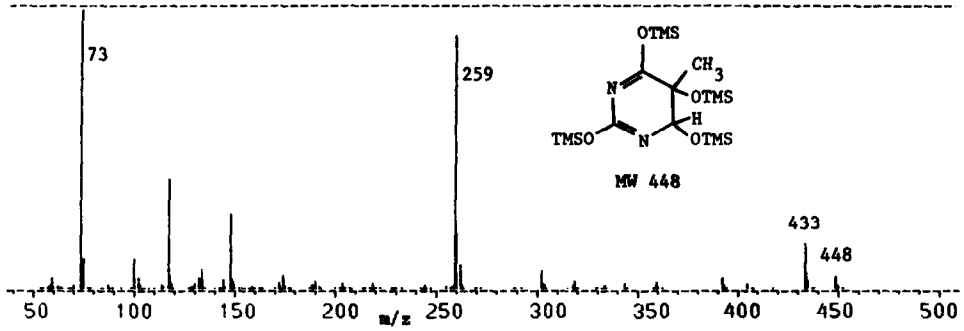

Fig. 5. Mass spectrum taken from peaks 8 and 9 in Fig. 1*.

which corresponds to loss of 189 a.m.u. from $M^{+\cdot}$. This ion however, could not be accounted for.

Peaks 10–20 in Fig. 1 represent TMS derivatives of radiation-induced dimeric products of thy[27]. The majority of the peaks, *i.e.*, peaks 15, 16 and those designated by 18 and 20 gave essentially identical mass spectra, one of which appears in Fig. 6. Products represented by these peaks as their TMS derivatives were assigned to the combination of OH adduct radicals of thy[27]. The insert in Fig. 6 shows one of the

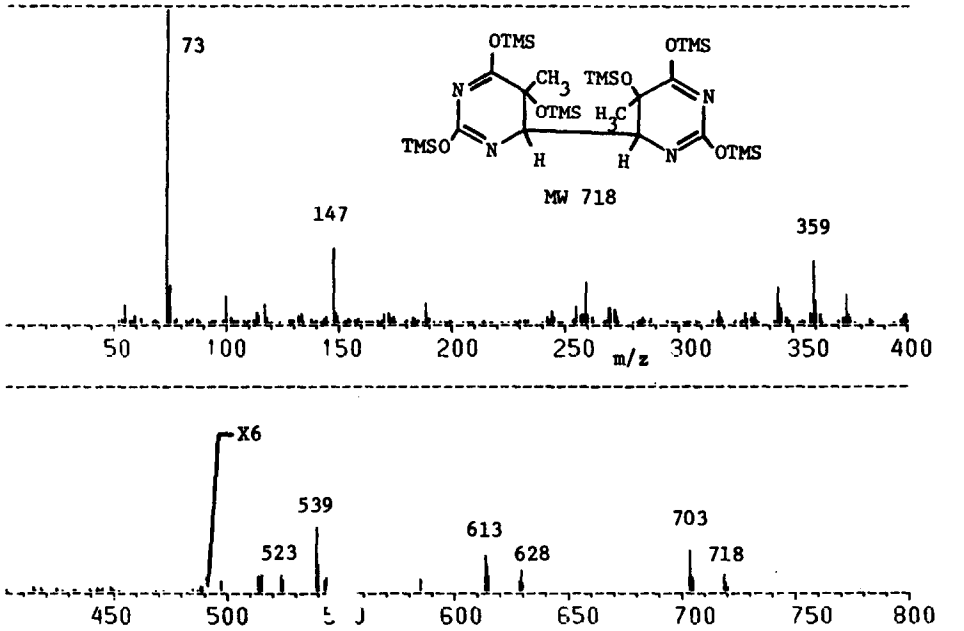

Fig. 6. Mass spectrum taken from peaks 15, 16 and those designated by 18 and 20 in Fig. 1.

* The second most intense ion at *m/z* 259 in Fig. 5 could not be accounted for. The interpretation of this mass spectrum was based on the $M^{+\cdot}$ and $(M-15)^+$ ions at *m/z* 448 and 433, respectively, and on the fact that thymine glycol is known to be the major radiation-induced monomeric product of thymine (see ref. 8).

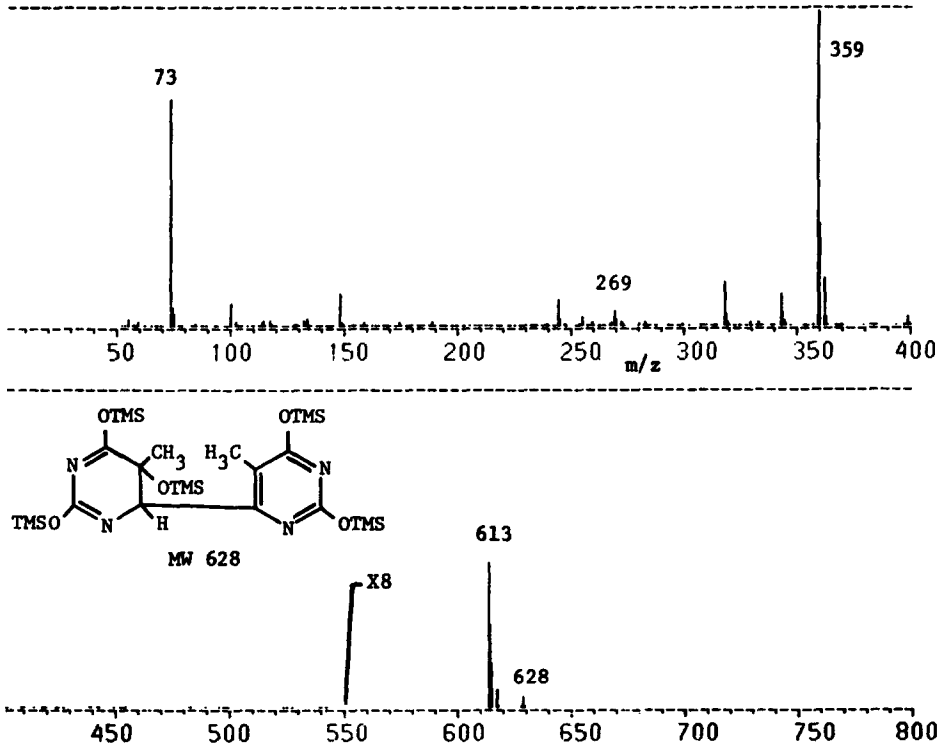

Fig. 7. Mass spectrum taken from peaks 12, 13, 17 and 19 in Fig. 1.

possible dimers representing various isomers. The large number of peaks was due to the combination of the same or different types of radiation-induced radicals of thy with the possible formation of various stereoisomers[27]. $M^{+\cdot}$ and $(M-15)^+$ were observed at *m/z* 718 and 703, respectively. Ions at *m/z* 628 and 613 were due to loss of HOTMS (90 a.m.u.) from $M^{+\cdot}$ and $(M-15)^+$, respectively. Elimination of OTMS from *m/z* 628 presumably accounted for the ion at *m/z* 539. Another characteristic ion was found at *m/z* 359, which represents cleavage of the molecule in half.

The mass spectra of peaks 12, 13, 17 and 19 were essentially identical (Fig. 7). $M^{+\cdot}$ and $(M-15)^+$ were observed at *m/z* 628 and 613, respectively. Other characteristic ions were at *m/z* 359 and 269. The insert shows the assigned structure. This compound was probably formed from some unstable isomers of the product whose TMS derivative is shown in Fig. 6 (or its isomers) by dehydration.

Peaks 10 and 11 gave identical mass spectra with ions at *m/z* 556 ($M^{+\cdot}$), 541 ($M^{+\cdot} - CH_3$), 467 ($M^{+\cdot} -$ OTMS), etc. $M^{+\cdot}$ and $(M-15)^+$ ions of these compounds show a difference of 72 a.m.u. from the corresponding ions of the former products (see insert in Fig. 7). This indicates that peaks 10 and 11 may represent some isomers of the compound shown in Fig. 7, but were not completely trimethylsilylated because of steric hindrances, and thus possess one free OH group.

Peak 14 in Fig. 1 gave a distinctive spectrum that appears in Fig. 8. The insert shows the assigned structure. This product was apparently formed by a further water elimination from the product (or its isomers), whose TMS derivative is shown in Fig.

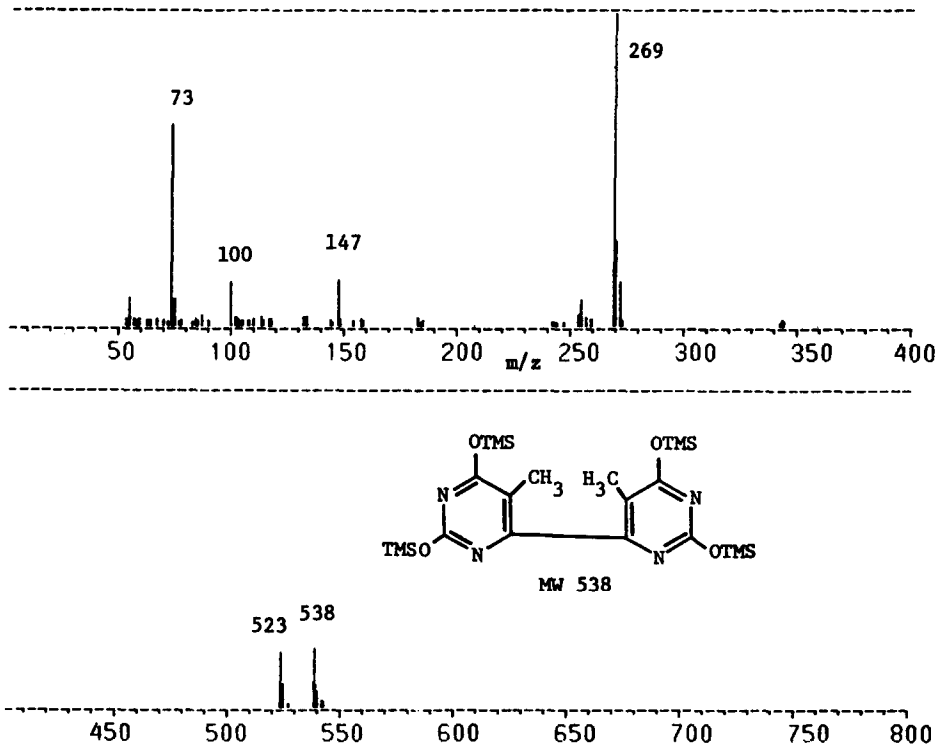

Fig. 8. Mass spectrum taken from peak 14 in Fig. 1.

7. Intense $M^{+\cdot}$ and $(M-15)^+$ were found at *m/z* 538 and 523, respectively. The ion representing cleavage of this molecule in half appears, surprisingly, as the base peak at *m/z* 269. Other peaks between peak 10 and 20 in Fig. 1 not discussed above correspond to mixtures of the dimers.

GC–MS analysis of γ-irradiated dT and pdT was carried out after hydrolysis with formic acid to remove the sugar or sugar–phosphate moiety. For comparison, a sample of γ-irradiated thy was also treated with formic acid and analyzed by GC–MS. In all cases similar chromatograms were obtained.

Products represented by the insert in Fig. 6 were still observed, but in decreased amounts, whereas an increase in the amounts of the products, whose mass spectra were shown in Figs. 7 and 8, was found. Other isomers of the compound shown in Fig. 8 are also possible, for instance such as the one with a $-CH_2-CH_2-$ bond between the two thymine moieties. This indicates that the water elimination mentioned above was an acid-catalyzed reaction.

GC–MS analysis of γ-irradiated cytosine, 2'-deoxycytidine and 2'-deoxycytidine-5'-monophosphate

A gas chromatogram obtained from trimethylsilylated γ-irradiated cyt is shown in Fig. 9. Peaks 2 and 3 represent TMS derivatives of cyt with one and two TMS groups attached to the amino group, respectively. Peaks 1 and 4–9 correspond to radiation-induced monomeric products of cyt[28]. As examples, mass spectra taken

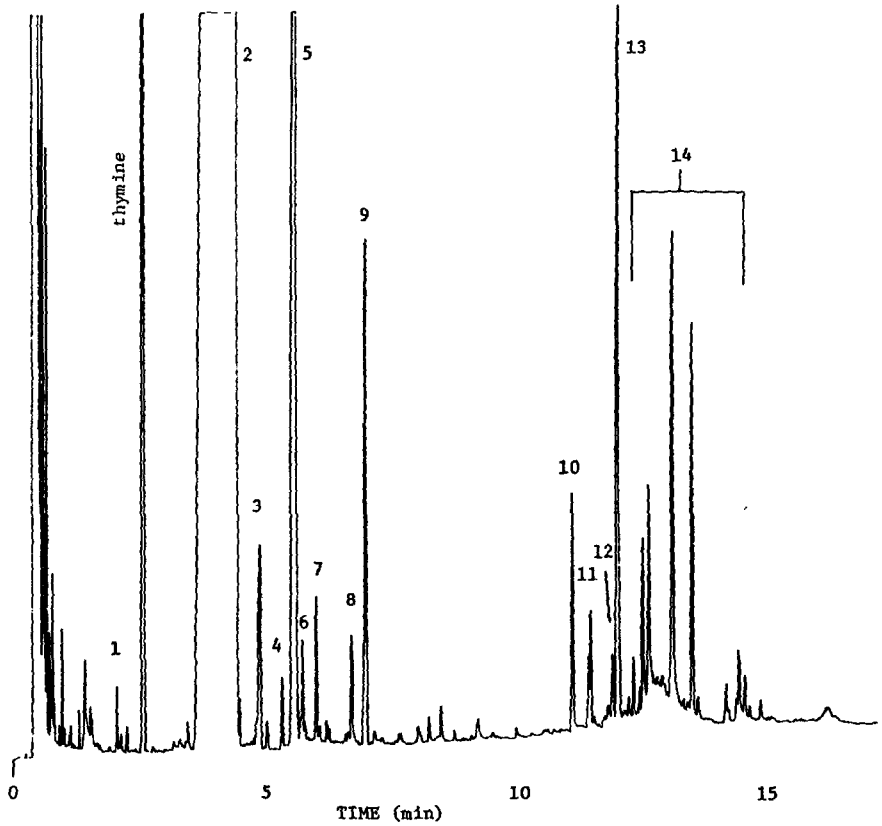

Fig. 9. Gas chromatogram obtained from a γ-irradiated sample of cytosine after trimethylsilylation. Column details as in Fig. 1. Thymine was added to the sample as an internal standard before derivatization.

from peaks 5 and 8 are shown in Figs. 10 and 11, respectively. Peak 5 was attributed to the TMS derivative of 5-hydroxycytosine (see insert in Fig. 10). Intense $M^{+\cdot}$ and $(M-15)^+$ were found at m/z 343 and 328, respectively, reflecting the aromatic character of this molecule. Peak 8 was ascribed to TMS derivative of 5,6-dihydroxy-5,6-dihydrocytosine (cyt glycol) (see insert in Fig. 11), and $M^{+\cdot}$ and $(M-15)^+$ were observed at m/z 433 and 418, respectively. The loss of OTMS from $M^{+\cdot}$ probably

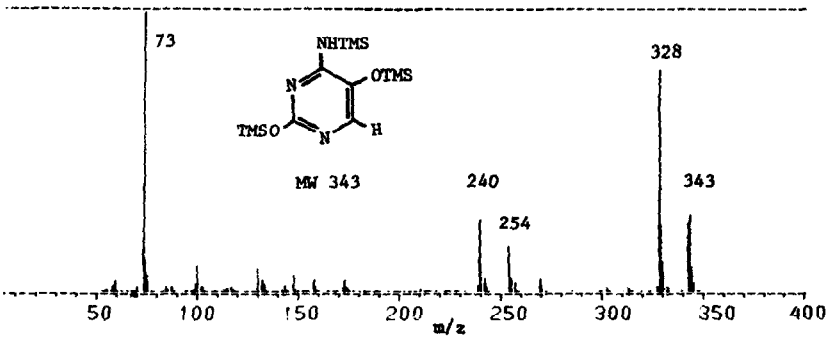

Fig. 10. Mass spectrum taken from peak 5 in Fig. 9.

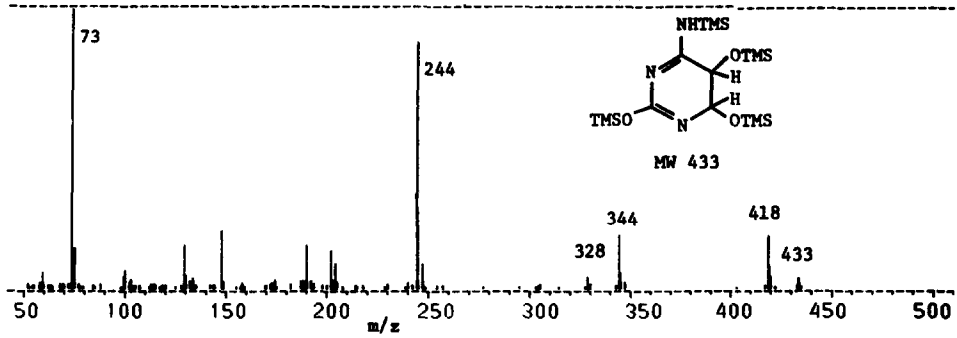

Fig. 11. Mass spectrum taken from peak 8 in Fig. 9.

accounted for the ion at m/z 344. The ion at m/z 328 was due to loss of HOTMS (90 a.m.u.) from $(M-15)^+$. As in the case of TMS thy glycol, an elimination of 189 a.m.u. from $M^{+\cdot}$ led to an intense ion at m/z 244.

Peaks 10–13 and those designated by 14 were attributed to TMS derivatives of radiation-induced dimeric products of cyt[28]. Essentially identical mass spectra were obtained from the majority of the peaks designated by 14, one of which appears in Fig. 12. Products represented by these peaks as their TMS derivatives were assigned to the combination of OH adduct radicals of cyt[28], and the structure shown in Fig. 12 is intended to represent various possible isomers including a number of stereoisomers[28]. $M^{+\cdot}$ was not observed, in this case, whereas $(M-15)^+$ was found

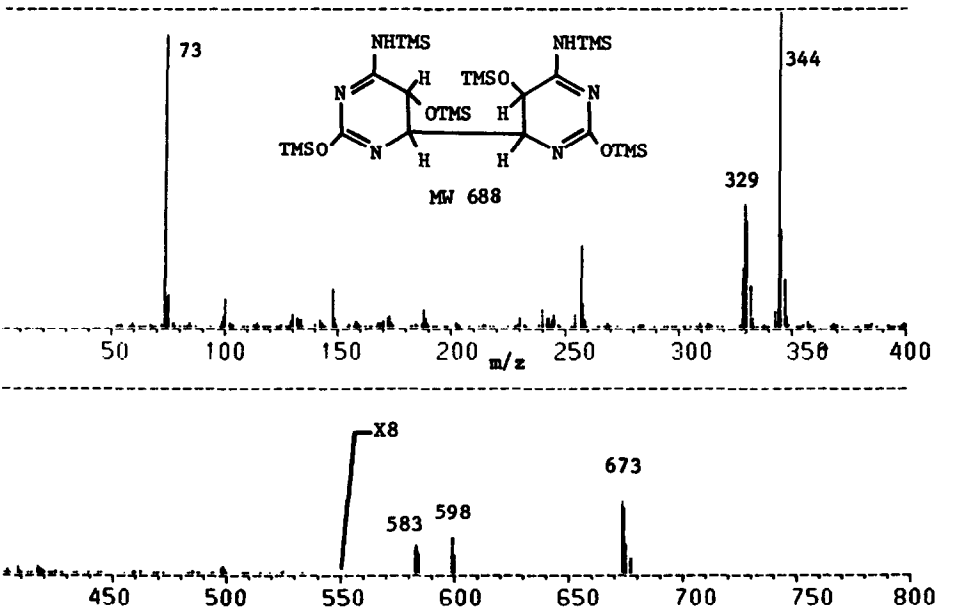

Fig. 12. Mass spectrum taken from peaks designated by 14 in Fig. 9.

* These compounds, although quite similar to the compound shown in Fig. 7, surprisingly showed a different fragmentation pattern.

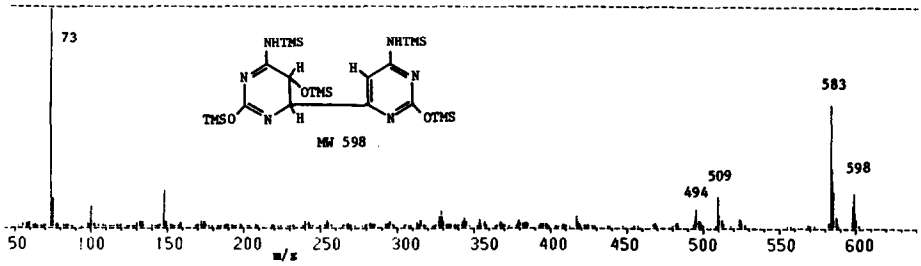

Fig. 13. Mass spectrum taken from peaks 11 and 13 in Fig. 9.

at m/z 673. The ions at m/z 598 and 583 were due to loss of HOTMS from $M^{+\cdot}$ and $(M-15)^+$, respectively. The m/z 344 ion representing cleavage of this molecule in half appeared as the base peak. Mass spectra taken from peaks 11 and 13 were identical (Fig. 13)*. The insert shows the assigned structure. These products were probably formed by dehydration of the product (or its isomers), whose TMS derivative is shown in Fig. 12. Intense $M^{+\cdot}$ and $(M-15)^+$ ions were found at m/z 598 and 583, respectively. Loss of OTMS from these ions obviously led to ions at m/z 509 and 494, respectively. A further water elimination probably accounted for the formation of the products represented by peaks 10 and 12 in Fig. 9. In their identical mass spectrum (Fig. 14), very intense $M^{+\cdot}$ and $(M-15)^+$ ions were observed at m/z 508 and 493, respectively, reflecting the aromatic character of this molecule.

GC–MS analysis of γ-irradiated dC and pdC was carried out after removal of the sugar or sugar–phosphate moieties by formic acid hydrolysis. Similar chromatograms were obtained from these samples and a sample of γ-irradiated cyt also treated with formic acid for comparison. The compound (or its isomers) shown in Fig. 12 was no longer observed whereas products shown in Figs. 13 and 14 were found.

GC–MS analysis of γ-irradiated mixtures of thymine, thymidine and thymidine-5'-monophosphate with tyrosine

A gas chromatogram obtained from an irradiated mixture of thy and Tyr after trimethylsilylation is given in Fig. 15. Peaks 1 and 10 represent TMS derivatives of thy and Tyr, respectively. Peaks 2–9 correspond to TMS derivatives of radiation-induced monomeric products of thy (see above), whereas peaks 11 and 12 represent TMS derivatives of 2- and 3-hydroxytyrosines, respectively, which are radiation-induced monomeric products of tyrosine[29].

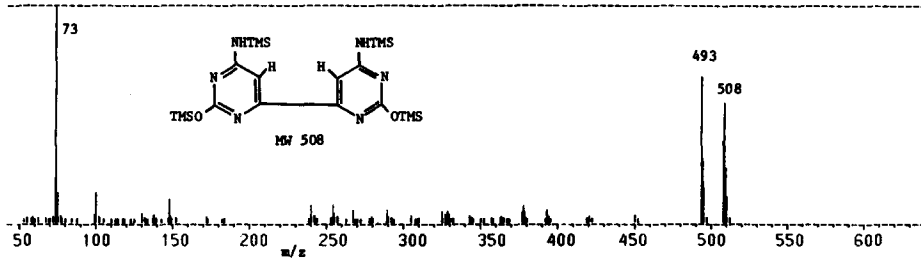

Fig. 14. Mass spectrum taken from peaks 10 and 12 in Fig. 9.

* These compounds, although quite similar to the compound shown in Fig. 7, surprisingly showed a different fragmentation pattern.

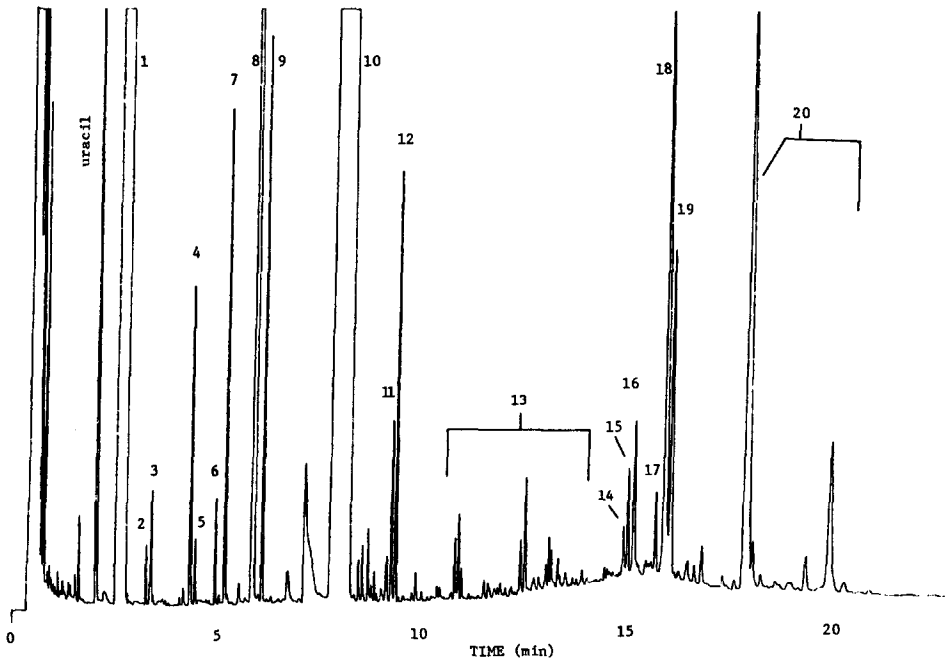

Fig. 15. Gas chromatogram obtained from a γ-irradiated mixture of thymine and tyrosine after trimethylsilylation. Column details as in Fig. 1. Uracil was added to the sample as an internal standard before derivatization.

Dimeric products of thy are represented by peaks designated by 13 (see above) and those of Tyr by peaks designated by 20 (ref. 29). Peaks 14–19 were attributed to radiation-induced crosslinks between thy and Tyr[30].

Essentially identical mass spectra were obtained from peaks 14, 15, 17 and 19 (Fig. 16). The insert shows the assigned structure, which represents a number of isomers. $M^{+\cdot}$ and $(M-15)^+$ ions were found at *m/z* 755 and 740, respectively. Loss of OTMS from $M^{+\cdot}$ led to an ion at *m/z* 666. Characteristic ions at *m/z* 638 and 538 were due to typical fragmentation of TMS derivatives of aromatic amino acids such as ($M^{+\cdot}$ – CO_2TMS) and ($M^{+\cdot}$ – 218 + H), respectively[31,32]. The ion at *m/z* 610 was probably formed from ion at *m/z* 638 by loss of CO. The loss of the amino acid side chain accounted for the intense ion at *m/z* 218. The mass spectrum taken from peak 16 appears in Fig. 17. Characteristic ions were observed at *m/z* 665 ($M^{+\cdot}$), 650 ($M^{+\cdot}$ – CH_3), 622 ($M^{+\cdot}$ – CH_3 – CO), 548 ($M^{+\cdot}$ – CO_2TMS), 520 (*m/z* 548 – CO), 448 ($M^{+\cdot}$ – 218 + H; base peak) and 218. All these ions showed a difference of 90 a.m.u. from the corresponding ions of the former products (see insert in Fig. 16). Thus, this compound (or its isomers) was probably formed from some isomers of the compound by water elimination, whose TMS derivative was shown in Fig. 16. The mass spectrum shown in Fig. 18 was obtained from peak 18 in Fig. 15. The insert shows the assigned structure. This compound was formed by combination of OH adduct radical of thy and a phenoxy radical derived from Tyr[30]. The $M^{+\cdot}$ (*m/z* 683) was not observed, whereas $(M-15)^+$ was found at *m/z* 668. Characteristic ions due to typical fragmentation of the amino acid moiety were observed at *m/z* 640

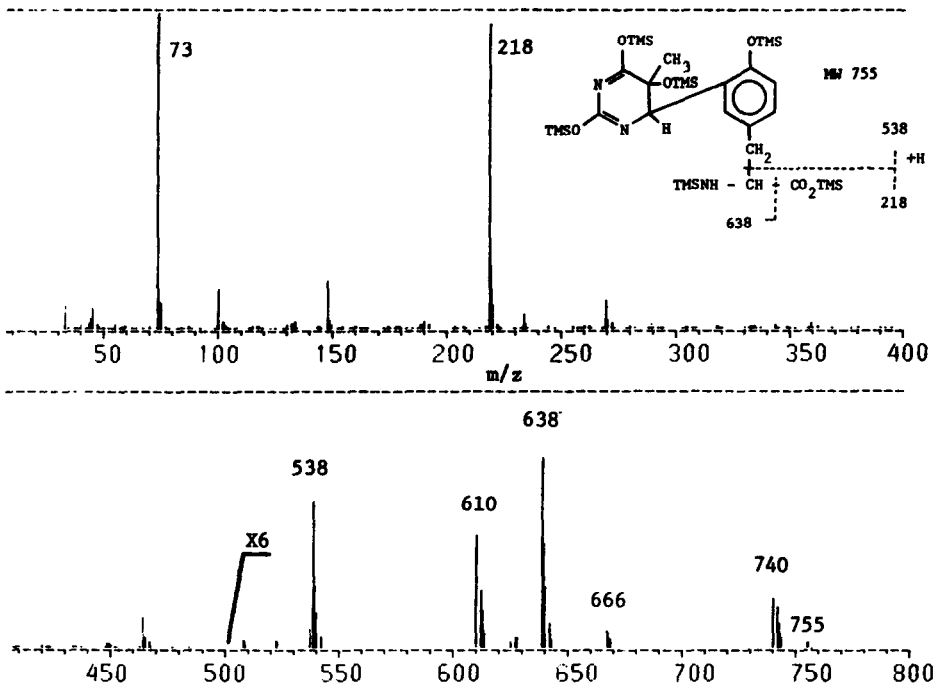

Fig. 16. Mass spectrum taken from peaks 14, 15, 17 and 19 in Fig. 15.

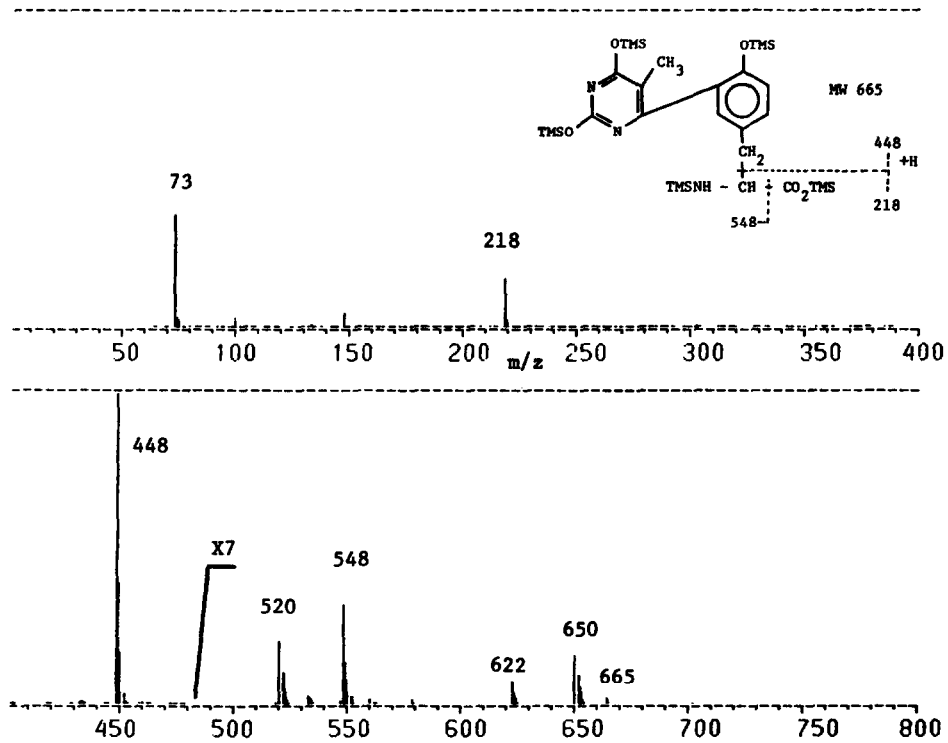

Fig. 17. Mass spectrum taken from peak 16 in Fig. 15. Isomers other than shown here are also possible.

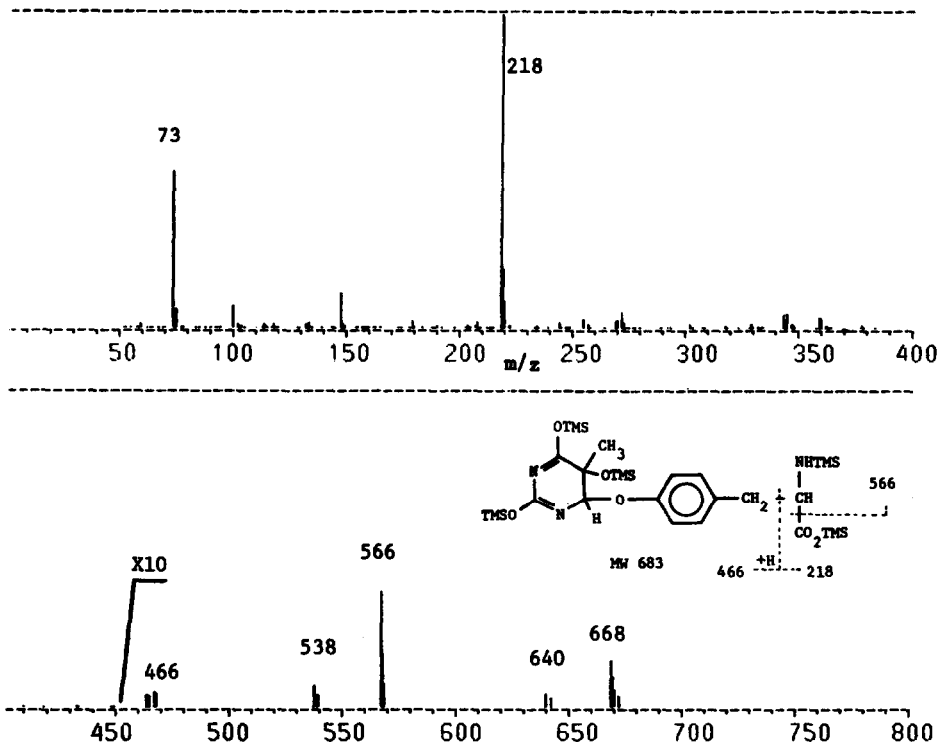

Fig. 18. Mass spectrum taken from peak 18 in Fig. 15. Isomers other than shown here are also possible.

($M^{+\cdot} - CH_3 - CO$), 566 ($M^{+\cdot} - CO_2TMS$), 538 (*m/z* 566 − CO), 466 ($M^{+\cdot}$ − 218 + H) and 218.

GC–MS analysis of irradiated mixtures of Tyr and dT or pdT was carried out after hydrochloric acid hydrolysis to remove the sugar moiety. Similar chromatograms were obtained from these samples and an irradiated mixture of thy and Tyr treated with hydrochloric acid. Products in Figs. 16 and 18 were still found, although in decreased amounts. An increase in the amount of the product in Fig. 17 was observed, indicating its formation by acid-catalyzed water elimination from the compound or its isomers, whose TMS derivative is shown in Fig. 16. In addition, another product, whose mass spectrum appears in Fig. 19, was observed. This product was probably formed by acid-catalyzed water elimination from some isomers of the product, whose TMS derivative is shown in Fig. 18. Characteristic ions found at *m/z* 593 ($M^{+\cdot}$), 578 ($M^{+\cdot} - CH_3$), 550 ($M^{+\cdot} - CH_3 - CO$), 476 ($M^{+\cdot} - CO_2TMS$), 448 ($M^{+\cdot} - CO_2TMS - CO$) and 376 ($M^{+\cdot}$ − 218 + H) present a difference of 90 a.m.u. (HOTMS) from the corresponding ions of the former compound. Other peaks between peaks 14 and 20 not discussed above represent mixtures of thy–Tyr cross-links.

Use of t-BDMS derivatives

In addition to TMS derivatives, the complementary use of t-BDMS derivatives may provide a highly useful means for identification of the products from radiation-

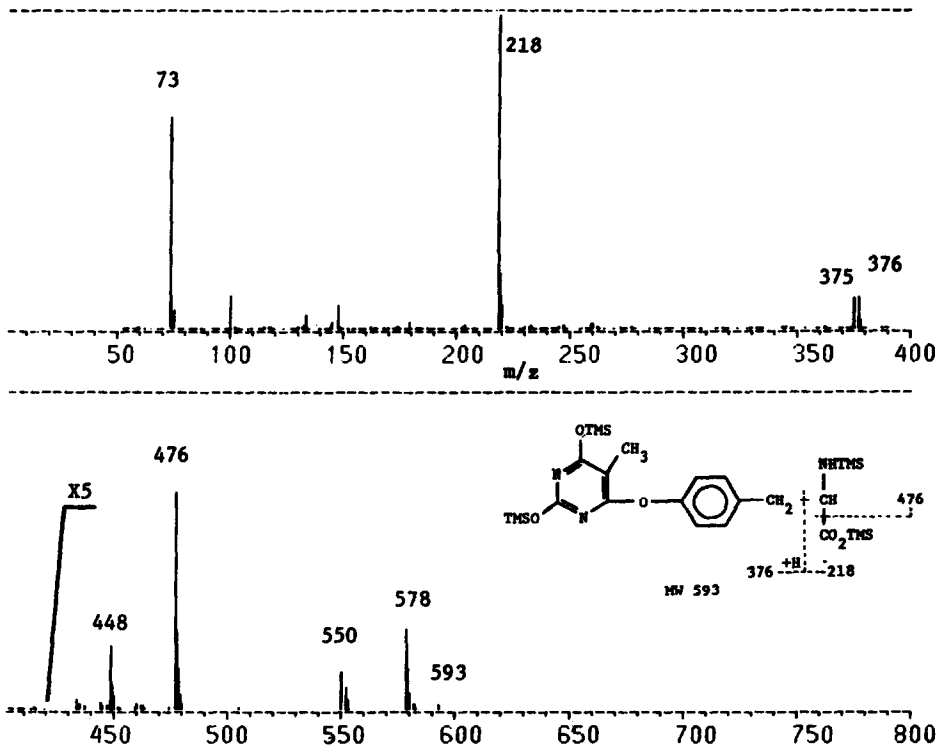

Fig. 19. Mass spectrum of a product found in HCl-hydrolyzed γ-irradiated mixtures of tyrosine and thymidine or thymidine-5'-monophosphate.

induced DNA base damage. These derivatives are also prepared in a simple procedure and have been shown to be very useful for MS analysis of nucleic acid bases[26,33]. The characteristic loss of the *tert.*-butyl radical (57 a.m.u.) from $M^{+\cdot}$ leads to a very abundant ion in the mass spectra[33] which may be used to easily determine the molecular weight and to monitor the corresponding compound in a mixture by means of selected-ion monitoring (SIM)[26].

In this work, t-BDMS derivatives of the radiation-induced products of thy were analyzed by GC–MS, as an example, to demonstrate the usefulness of this

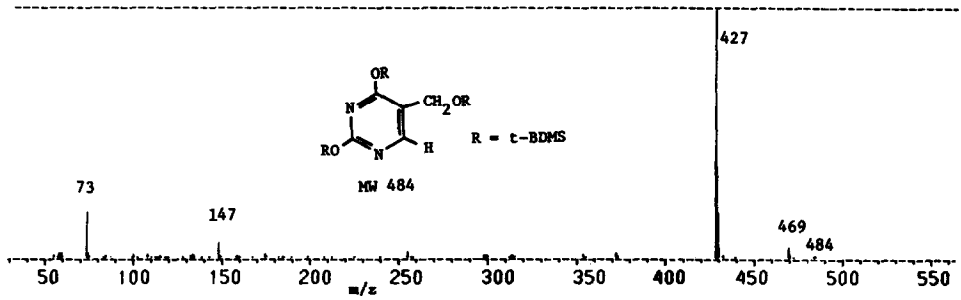

Fig. 20. Mass spectrum of t-BDMS derivative of 5-hydroxymethyluracil.

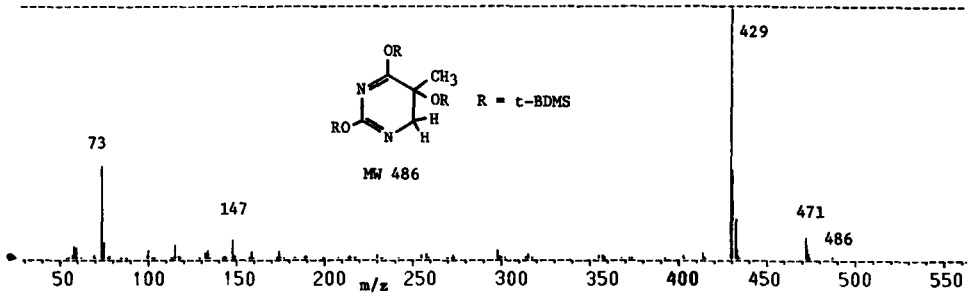

Fig. 21. Mass spectrum of t-BDMS derivative of 5- or 6-hydroxy-5,6-dihydrothymine.

method for identification of radiation-induced DNA base damage. Mass spectra of t-BDMS derivatives of 5-hydroxymethyluracil, 5- or 6-hydroxy-5,6-dihydrothymine, thy glycol and a dimeric product are given in Figs. 20–23. In the case of the first two compounds (Figs. 20 and 21), the $(M-57)^+$ ion appeared as the base peak at m/z 427 and 429, respectively. $M^{+\cdot}$ and $(M-15)^+$ were also observed. In the mass spectrum of t-BDMS derivatives of thy glycol (Fig. 22), $(M-57)^+$ was found as the second most abundant ion at m/z 559. $M^{+\cdot}$ and $(M-15)^+$ were also present at m/z 616 and 601, respectively.

The $(M-57)^+$ ion was also the second most abundant ion at m/z 781 in the mass spectrum of the dimeric product shown in Fig. 23. The high intensity ($\approx 32\%$ relative intensity) of an ion in that region of high a.m.u. is very unusual for TMS

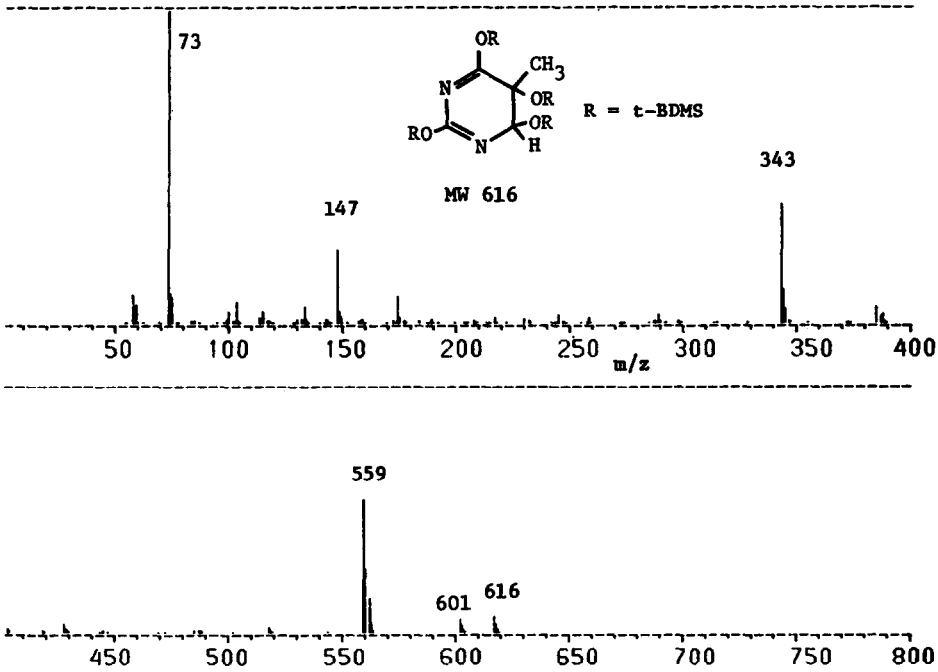

Fig. 22. Mass spectrum of t-BDMS derivative of thymine glycol.

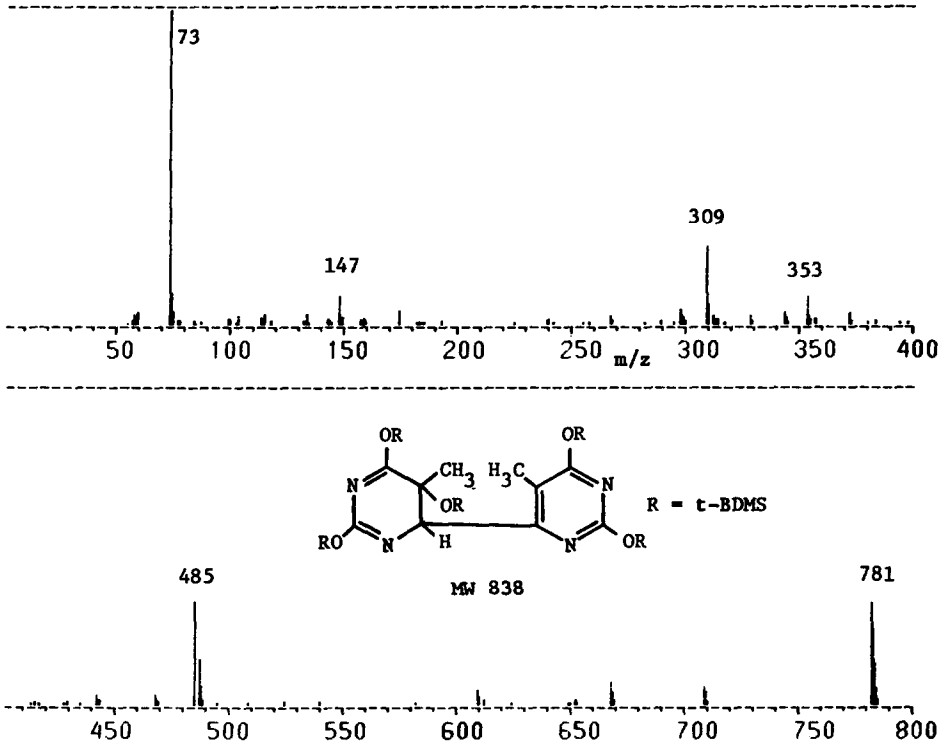

Fig. 23. Mass spectrum of t-BDMS derivative of a radiation-induced dimeric product of thymine. Isomers other than shown here are also possible.

derivatives, indicating the extra usefulness of t-BDMS derivatives for such diagnostic purposes. Ions with higher a.m.u. than *m/z* 781 could not be observed, because the instrument used monitors ions only up to 800 a.m.u.

Comments

Most of the compounds, especially the dimeric products, investigated in this work are very labile and tend to decompose on the column. Thus, the choice of the column is a crucial factor for a successful GC–MS analysis (as always). Of the fused-silica capillary columns coated with different stationary phases such as OV-101, OV-1, SP-2100 and SE-54, columns coated with cross-linked SE-54 (narrow or wide bore) yielded the best results.

As would be expected, narrow bore columns (0.2 mm I.D.; 0.11 μm film thickness) coated with cross-linked SE-54 showed better efficiency (n = 5400 plates per meter), thus better resolution, than wide bore columns coated with the same stationary phase (0.3 mm I.D.; 0.17 μm film thickness; n = 3500 plates per meter). On the other hand, wide bore columns offer a higher loading capacity than narrow bore columns. Moreover, the length of the column was found to be an important factor because the dimeric products examined have readily decomposed on columns longer than 10–12 m. In most instances, no dimeric products were observed when 25-m columns were used. In addition to the column length, analysis time was found to

play an important role in decomposition of the compounds tested here. Degradation of these compounds was minimized by keeping analysis times as short as possible without sacrificing resolution (15–20 min).

When a column was used for the first time or after a period of storage, it was primed with a 1-μl injection of BSTFA in the splitless mode to cap any active sites that may have been formed during nonuse periods. Then, injections of the samples were made in the split mode.

The method described here provides high sensitivity. A signal-to-noise ratio of 2, which is the minimum detection limit by definition, was found to correspond to *ca.* 0.1 pmol of standard compounds such as thymine, cytosine, or tyrosine. It was concluded that amounts as low as 1 pmol of altered DNA bases can be conveniently detected by this method.

Life of the columns used in this work was found to be typically on the order of 3–6 months, depending on frequency of use and amounts of injected materials. Diminished sensitivity, tailing peaks or decomposition of dimeric products are signs of a deteriorating column.

CONCLUSIONS

The results presented here clearly show that capillary GC–MS is well suited for separation and identification of radiation-induced DNA base damage and DNA base–amino acid crosslinks. The TMS derivatives of the compounds, which have been examined here, have good GC properties and easily interpretable mass spectra. The presence of the molecular ion and a characteristic $(M-CH_3)^+$ ion in the mass spectra facilitates structural elucidation. In addition, the complementary use of t-BDMS derivatives constitutes a highly useful means for identification by providing an intense characteristic $(M-57)^+$ ion in mass spectra, which may be used to monitor the types of compounds examined here in a complex mixture by means of the SIM technique. Since there are DNA repair enzymes such as glycosylases[34], which recognize and selectively remove damaged bases from DNA, the methodology described here can usefully be applied to investigation of the repairability of damaged sites of DNA and to identification and quantitation of the damaged bases removed by repair enzymes. It is plausible that the methodology described may be applicable not only to identification of radiation-induced DNA base damage, but also to that of DNA base damage caused by agents other than radiation.

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