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ANALYSIS OF CYTOSINE ARABINOSIDE AND RELATED PYRIMIDINE NUCLEOSIDES BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Acetyl methyl derivatives of cytosine arabinoside and six other pyrimidine nucleosides were prepared and examined by gas–liquid chromatography (GLC) using a nitrogen-sensitive flame ionization detector and by gas chromatography–mass spectrometry (GC–MS). The derivatives gave symmetrical GC peaks and could be separated on SE-30 or OV-17. Detection limit for cytosine arabinoside was approximately 500 pg using both the nitrogen-sensitive flame ionization detector and single-ion monitoring $[(M - 15)^+$ ion]. Methyl trimethylsilyl and three homologous alkyl oxime trimethylsilyl derivatives of cytosine arabinoside and cytidine were also prepared and examined by GC–MS. Retention indices are reported on four phases and the mass spectra of these derivatives discussed. The methyl trimethylsilyl derivatives gave a lower detection limit than the methyl acetyl derivatives (approximately 50 pg), but the oxime derivatives were less sensitive. Allyl and propyl dimethylsilyl derivatives of cytosine arabinoside were also examined.

INTRODUCTION

The arabinonucleosides are of biological interest because of their potent antiviral and antitumour activity. In particular, cytosine arabinoside (1-β-D-arabinofuranosylcytosine, ara-C) is one of the most effective drugs for treatment of acute myeloblastic leukaemia in man. As part of a study of the pharmacokinetics of ara-C in man, physical methods for analysis of this compound were investigated. This report examines the application of gas–liquid chromatography and gas chromatography–mass spectrometry (GC–MS) for specific and sensitive detection of ara-C with separation from its major metabolite uracil arabinoside (ara-U) and from the closely related naturally occurring ribo- and deoxyribo-pyrimidine nucleosides.

In the past, purine and pyrimidine nucleosides have presented a difficult application to GC because of their high polarity. Yet, GC has the potential for being a rapid and sensitive analytical technique, especially since nucleosides contain a relatively high proportion of nitrogen atoms which will give a favourable response to a nitrogen-sensitive flame ionization detector (FID). The first derivatives applicable to GC of nucleosides were acetates[1]. These chromatographed to give broad peaks and have not been subsequently applied to biomedical problems involving nucleoside analysis. Trimethylsilyl (TMS) derivatives have been investigated to a greater extent recently[2-8] but there has been only limited application to chemotherapeutic and clinical investigation[9-11]. Furthermore, while TMS derivatisation of some nucleosides is relatively simple, those containing an amino group are more difficult to form and in some cases more than one derivative is obtained[7]. Cytidine, the 2'-hydroxy epimer of ara-C, for example, gives a broad peak with significant tailing, whilst deoxycytidine has so far produced no chromatographic derivatives after trimethylsilylation[5,6]. To overcome this problem Butts[12] showed that chromatographically better results were obtained when the amino group of cytidine and deoxycytidine was first converted to a methoxime followed by trimethylsilylation of the sugar. In keeping with this approach, the chromatographic analysis of ara-C was investigated using double derivatives to increase volatility. These included methyloxime (MO), ethyloxime (EO) and n-butyloxime (BuO) TMS derivatives, and the acetates, and various alkylsilyl derivatives of nucleosides whose bases had been methylated with diazomethane. Detection by a nitrogen-sensitive FID gave greatly enhanced sensitivity over a flame detector but in plasma, high sensitivity and selectivity was best achieved using combined GC–MS in either the single- or multiple-ion mode (results to be published).

EXPERIMENTAL

Materials

Ara-C was donated by Upjohn (Crawley, Great Britain). Ara-U was obtained from Calbiochem (San Diego, Calif., U.S.A.). 2'-Deoxycytidine and 2'-deoxyuridine were obtained from BDH (Poole, Great Britain). Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Trimethylchlorosilane (TMCS) was obtained from Pierce (Rockford, Ill., U.S.A.). Methoxyamine hydrochloride and ethoxyamine hydrochloride were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Butoxyamine hydrochloride was obtained from Applied Science Labs. (State College, Pa., U.S.A.). Allyl and propyl dimethylchlorosilane were supplied by Pfaltz & Bauer (Stamford, Conn., U.S.A.) and Field Instr. (Twickenham, Great Britain), respectively. Cytidine, uridine and thymidine were obtained from Sigma (St. Louis, Mo., U.S.A.). Ethereal diazomethane was prepared from Diazald (Aldrich). Acetonitrile was from BDH; acetic anhydride (AR) was from Fisons (Loughborough, Great Britain) and pyridine was redistilled and stored over KOH. Catalyst solution of boron trifluoride was prepared by diluting 100 μl of boron trifluoride etherate (BDH) with 5 ml of diethyl ether.

Derivatization

Stock solutions of the nucleosides were prepared in methanol at a concentration of 1 mg/ml. Aliquots were removed and evaporated to dryness under a stream

of nitrogen in an all-glass cone-shaped test tube to give the required quantities for derivatization.

Acetyl methyl derivatives. The nucleoside (100 μg) was acetylated by treatment with acetic anhydride (60 μl) and pyridine (30 μl) at room temperature for 40 min. The excess reagent was evaporated to dryness at 60° under a stream of nitrogen and the residue was dissolved in ethyl acetate (50 μl) and was methylated as described below.

The acetylated nucleosides containing the uracil or substituted uracil base (ara-U, deoxyuridine, uridine and thymidine) were treated with ethereal diazomethane (200–400 μl) and methylation was complete after approximately 60 sec. The excess reagent and solvent were evaporated under a stream of nitrogen and the residue was dissolved in ethyl acetate (100 μl) for GC analysis.

The acetylated derivatives of the amino nucleosides (ara-C, cytidine, deoxycytidine) were treated with ethereal diazomethane (200–400 μl) followed by addition of 2–3 μl of catalyst solution of boron trifluoride etherate in diethyl ether containing approx. 0.04–0.07 μg borontrifluoride etherate. Further ethereal diazomethane was added if necessary to maintain excess diazomethane. After the addition of the boron trifluoride, methylation was complete after approx. 60 sec and the mixture was evaporated to complete dryness under a stream of nitrogen and the residue was dissolved in ethyl acetate (100 μl) for GC analysis.

Trimethylsilyl dimethyl derivatives. The nucleoside (100 μg) was silylated by treatment with acetonitrile (100 μl), BSTFA (100 μl) and TMCS (5 μl) at 100° for 30 min. The excess reagent was removed by evaporation under a stream of nitrogen. The residue containing the TMS derivative was then dissolved in ethyl acetate (50 μl) and ethereal diazomethane (200–300 μl) was added, followed by slow addition of 4–5 μl of boron trifluoride etherate in diethyl ether. Further ethereal diazomethane was added if necessary to maintain excess diazomethane in the mixture. The yellow solution was then left at room temperature for 10 min to complete the methylation and then was evaporated to complete dryness under a stream of nitrogen. The residue was redissolved in ethyl acetate (100 μl) for GC analysis.

Propyl and allyl dimethylsilyl methyl derivatives. The nucleoside (100 μg) was treated with propyl dimethylchlorosilane or allyl dimethylchlorosilane (100 μl), acetonitrile (100 μl) and diethylamine (50 μl) and the mixture was heated at 100° for one hour. After centrifugation to remove the amine salt, the supernatant was transferred to another tube and evaporated to near dryness at 70° under a stream of nitrogen. The residue was dissolved in ethyl acetate (100 μl) and methylated with diazomethane and boron trifluoride as described above.

Trimethylsilyl alkoxime derivatives. Pyridine (200 μl) containing 25 mg/ml of methoxyamine hydrochloride, ethoxyamine hydrochloride or butoxyamine hydrochloride was added to the nucleoside (100 μg) and the mixture was heated at 80° for 4 h to complete derivatization. The mixture was then evaporated to dryness at 60° under a stream of nitrogen. The oxime derivative was then silylated by treatment with acetonitrile (100 μl), BSTFA (100 μl) and TMCS (5 μl) at 100° for 30 min. The excess silylation reagent was evaporated under a stream of nitrogen and the residue was dissolved in ethyl acetate (100 μl) for GC analysis.

Deuterated derivatives. Me-[^{2}H$_9$]TMS derivatives were prepared from ara-C by treating the methylated nucleoside (prepared by treating 1 mg/ml methanolic solu-

tion of ara-C with ethereal diazomethane and boron trifluoride as described above for 10 min at room temperature)with [$^2H_{18}$] bis(trimethylsilyl)acetamide ([$^2H_{18}$]BSA) and a trace of TMCS for 30 min at 100°. [$^2H_3$]acetate and [$^2H_3$]methyl analogues of the methyl acetyl derivative of ara-C were prepared by substituting [$^2H_6$]acetic anhydride and [$^2H_2$]diazomethane for the unlabelled reagents in the above preparations.

Extraction of ara-C from plasma

Plasma (1 ml) was mixed with 15% (v/v) trichloroacetic acid (3 ml) and stood in ice for 30 min to complete protein precipitation. The mixture was centrifuged for 10 min, shaken four times with 3-ml volumes of ether (the ether was discarded) and once with iso-amyl alcohol. The aqueous phase was freeze dried and derivatized as described above.

Gas chromatography

Retention indices of the derivatives of ara-C and cytidine were measured on 3% SE-30, 3% OV-17, 3% OV-25 and 3% OV-210, all on 100–120 mesh Gas-Chrom Q (Applied Science Labs.) and packed into 2 m × 2 mm I.D. glass columns. These were fitted to a Varian 2400 gas chromatograph with FIDs. Nitrogen at 30 ml/min was used as the carrier gas. The injector and detector oven temperatures were maintained at 280° and 300°, respectively, and the column oven was kept at temperatures from 200° to 250° depending on the retention time of the derivative. All other GC work was performed on a Hewlett-Packard 5750G gas chromatograph with a nitrogen–phosphorus-specific FID (N-FID) and temperature programme. The chromatograph was linked to a Hewlett-Packard 3370 digital integrator for chart presentation and peak area determination. Glass columns (2 m × 4 mm I.D.) were packed with 3% SE-30 on 80–100 Chromosorb W (Pierce) or 3% OV-17 on 100–120 Gas-Chrom Q. The injection port temperature was 290° and the N-FID was maintained at 400°. Column oven temperatures were as indicated below in the text. Flow-rates were: helium (carrier gas) 45 ml/min, hydrogen 28 ml/min and air 180 ml/min. The height of the rubidium bromide crystal in the detector block was adjusted to give equal response to a 2-μl injection of azobenzene (10 ng) and octadecane (5000 ng) in hexane when the instrument was in the "normal" mode. For all subsequent analysis the instrument was used in the "high sensitive" mode.

Gas chromatography–mass spectrometry

Low-resolution mass spectra were recorded with a V.G. Micromass 12B mass spectrometer interfaced to a V.G. Data system type 2040 and via a glass jet separator to a Varian 2400 gas chromatograph. The GC column was 2 m × 2 mm 3% SE-30 on Gas-Chrom Q with helium at 30 ml/min as the carrier gas. The spectrometer ion-source temperature was 260° and the accelerating voltage 2.5 kV. Spectra were recorded at 25 eV with a scan speed of 3 sec per decade.

Single- and multiple-ion monitoring were performed with a V.G. Micromass 70/70F mass spectrometer interfaced to a Varian 2400 gas chromatograph using 1 or 2 m × 2 mm I.D. columns packed with 3% SE-30 or 3% OV-17. The column oven temperature was set to give a retention time of 1.5 min (in the range 230° for 1 m SE-30, to 265° for 2 m OV-17). The mass spectrometer was operated at 70 eV

with an accelerating voltage of 4 kV and a trap current of 100 μA. GC interface and ion-source temperatures were 300° and 260°, respectively.

RESULTS AND DISCUSSION

The acetyl methyl derivatives were readily prepared and required no elevated temperatures or standing for several hours to complete the reaction. Acetylation was employed because of the relative speed of the reaction with acetic anhydride–pyridine at room temperature and that the acetylation mixture was sufficiently volatile to be easily removed by evaporation. Furthermore the acetates were stable in aqueous environment and partitioned favourably into an organic solvent (chloroform or ethyl acetate). This is important when biological extracts require further purification after acetylation. These nucleosides containing the uracil base were acetylated only on the sugar moiety and there was no change in the ultraviolet spectrum to that of the parent nucleoside (λ_{max} in 0.1 N HCl for acetylated ara-U, uridine, deoxyuridine, thymidine was 261 nm) whilst the amino nucleosides gave acetylation of the amino group with corresponding absorption at longer wavelength (λ_{max} for acetylated ara-C, cytidine, deoxycytidine was 310 nm). Both classes of compounds chromatographed on either 3 % SE-30 or 3 % OV-17 stationary phases to give broad peaks with extensive tailing and which were not suitable for analysis (Figs. 1 and 2). Deoxycytidine acetate, as with its TMS derivative[5,6] did not chromatograph (Fig. 3).
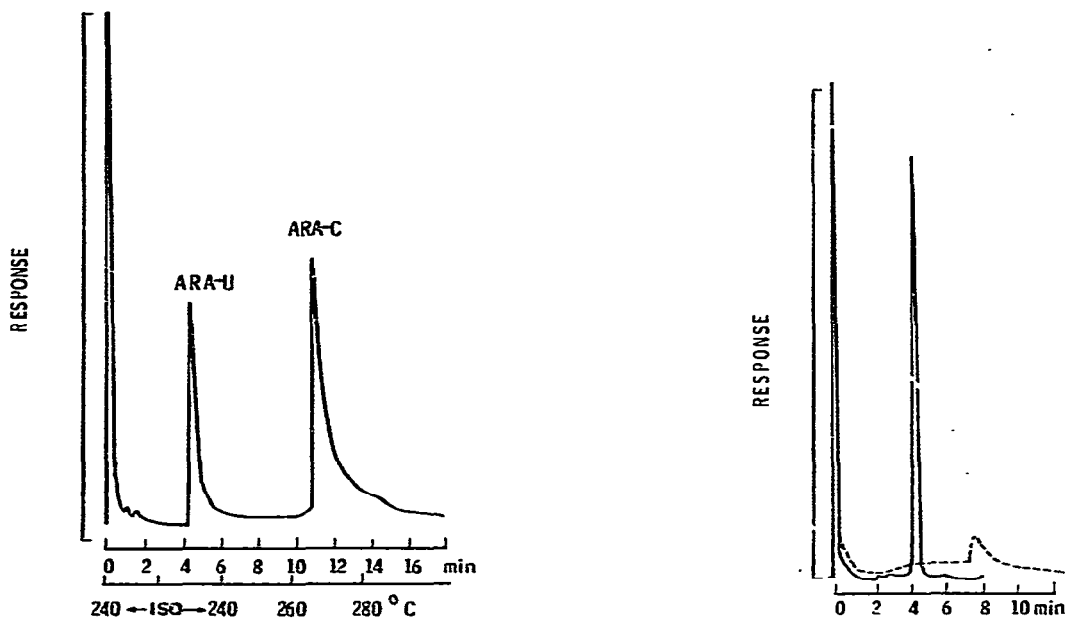

Fig. 1. Gas chromatogram of ara-C and ara-U as acetyl derivatives on 3 % SE-30 column. Temperature, 240° for 6 min then programmed at 6°/min.

Fig. 2. Gas chromatogram of cytidine as acetyl derivative (broken line) and acetyl methyl derivative (solid line). Column, 3 % SE-30. Temperature, isothermal at 260°.
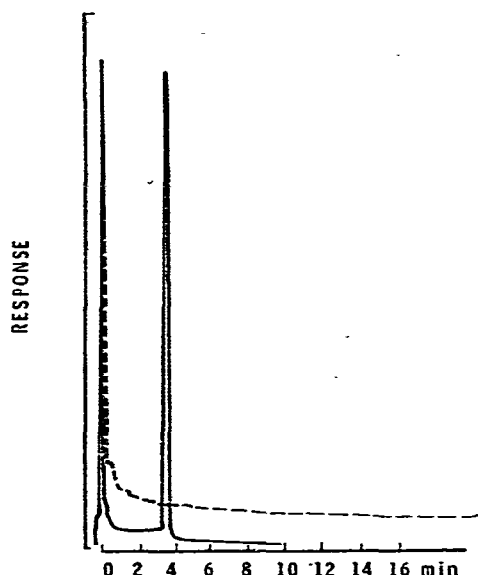
Fig. 3. Gas chromatogram of deoxycytidine as acetyl derivative (broken line) and acetyl methyl derivative (solid line). Column, 3% SE-30. Temperature, isothermal at 260°.

Reaction of acetylated derivatives with ethereal diazomethane gave the acetyl methyl derivative which had correspondingly superior chromatographic properties over the acetates (Figs. 2 and 3). The uracil nucleosides reacted almost instantaneously with ethereal diazomethane, whilst with the amino nucleosides the reaction was incomplete with diazomethane alone giving approximately 30% methylation with little further change on standing at room temperature for up to 30 min. However addition of small amounts of boron trifluoride etherate as catalyst[13],[14] greatly enhanced the speed of reaction and methylation was complete after 1–2 min. The ultraviolet spectrum for ara-C or cytidine showed $\lambda_{max.}$ at 280 nm (same as for the parent nucleoside) indicating methylation of the amino group. The mass spectrum of ara-C as the methyl acetyl derivative is shown in Fig. 4 and is consistent with the structure shown. The characteristic ions of highest mass were the low abundance molecular ion (m/e 425) and $(M - 15)^+$ (m/e 410). The main fragments at lower mass arose from cleavage of the derivatised sugar yielding a similar pattern to that of permethylated ara-C[15]. Shifts in the spectra of the deuterated analogues confirmed the fragmentation shown. The mass spectrum of the acetyl methyl derivative of cytidine was similar to that of the acetyl methyl derivative of ara-C. The mass spectrum of the acetyl methyl derivative of deoxycytidine contained a molecular ion [m/e 367 (0.8%)] and an M — 15 [m/e 352 (0.9%)] ion in low abundance. The ions containing the base [m/e 168 (11%), 167 (7%), 152 (44%)] were more abundant than in the spectra of the corresponding derivatives of ara-C and cytidine. The sugar fragment [m/e 201 (2.7%)] was weaker but fragmented with loss of two molecules of acetic acid to give the base peak (m/e 81, C_5H_6O).

Chromatographic separation of the acetyl-methyl derivatives of the nucleosides was effected on 3% SE-30 as shown in Fig. 5. Of significance is the complete separa-
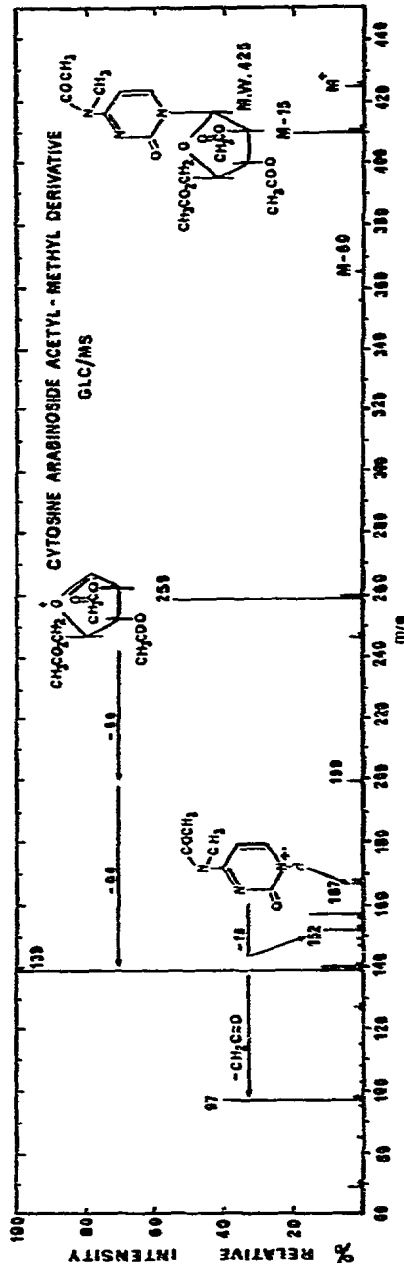
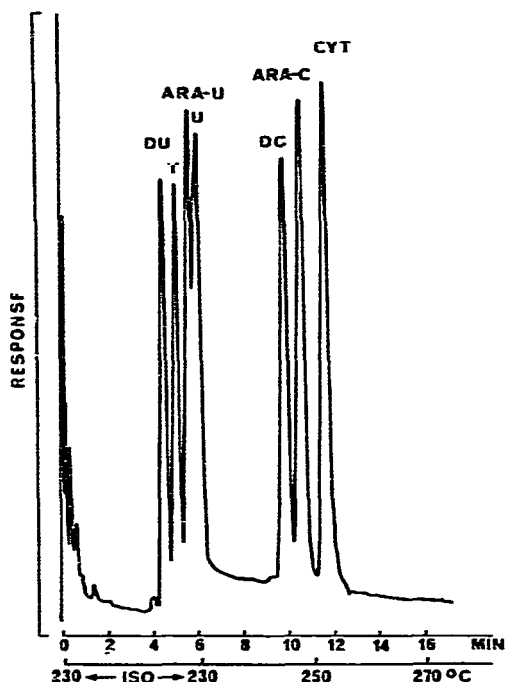Fig. 4. 25-eV mass spectrum of the acetyl methyl derivative of ara-C.

Fig. 5. Gas chromatogram of acetyl methyl derivatives of deoxyuridine (DU); thymidine (T); ara-U, uridine (U); deoxycytidine (DC); ara-C and cytidine (CYT). Column, 3 % SE-30. Temperature, 230° for 6 min then programmed at 4°/min. Sample size, 1 μl containing derivatives from 1 μg (free base) in ethyl acetate. Instrument settings: range, 10³; attenuation, 64.

tion of ara-C from ara-U, as well as separation of ara-C from deoxycytidine and cytidine. This is important if analysis of ara-C in biological fluids is to be free from interference from these endogenous nucleosides. Similar separation of the nucleosides was achieved on 3 % OV-17, and 3 % OV-25 but the separation on OV-210 was poor. Retention indices for these and other derivatives are given in Table I. The corresponding purine ribonucleoside analogues, adenosine and inosine, when derivatised in identical manner did not yield chromatographic derivatives under the conditions used.

For quantitative analysis the acetyl methyl derivative of ara-C showed linear detection response (peak height or peak area) with quantities up to 1 μg, with a minimum detectable quantity of less than 0.5 ng using both the nitrogen detector and single-ion monitoring. For the latter, the $(M - 15)^+$ ion (m/e 410) was used because, although it gave a lower absolute sensitivity than the more abundant ions such as the base peak (m/e 139), when monitored in plasma[16], poor signal-to-noise ratios were obtained with the low-mass ions because of interferences by endogenous plasma constituents.

The low abundance of ions at high mass in the spectrum of the acetyl methyl derivative of ara-C prompted the investigation of other derivatives whose mass spectra contained ions of higher abundance at high mass and which would thus possibly give enhanced sensitivity. The spectrum of the tris-TMS derivative of ara-C did not contain a suitable ion. The $(M - CH_3)^+$ at m/e 444 was the ion of highest mass

TABLE I

RETENTION INDICES OF THE DERIVATIVES OF ara-C AND CYTIDINE

Compound	Derivative	3% SE-30	3% OV-17	3% OV-25	3% OV-210
Ara-C	Me-Ac	2720	3247	3484	4265
	Me-TMS	*	2585	2672	3045
	Me$_2$-TMS	2433	2572	2655	2944
	MO-TMS	2455	2608	2693	3075
	EO-TMS	2518	2650	2740	3126
	BuO-TMS	2715	2843	2918	3325
	Me$_2$-ADMS	2907	3115	—	—
	Me$_2$-PrDMS	2896	—	—	—
Cytidine	Me-Ac	2770	3305	3558	4250
	Me-TMS	*	2620	2730	3083
	Me$_2$-TMS	2435	2606	2700	2935
	MO-TMS	2503	2643	2735	3088
	EO-TMS	2545	2685	2790	3150
	BuO-TMS	2734	2882	2962	3322

* Did not separate from the Me$_2$TMS derivative.

observed but its abundance was only 4%. Also this compound produced a peak with extensive tailing, similar to that of the TMS derivative of cytidine[6,12]. Methylation of the TMS derivative in the presence of BF$_3$ gave, after a few minutes, a mixture of mono- and dimethyl derivatives and complete conversion to the dimethyl-TMS derivative was achieved after 10 min. By varying both the quantity of BF$_3$ and the time, it was possible to prepare either derivative in the pure state. Trimethylsilylation was achieved by heating the nucleoside with BSTFA and TMCS in acetonitrile for 30 min at 100°, a method similar to that reported to give optimum yields for various nucleosides[6,7].

Table I gives the retention indices for the mono- and dimethyl derivatives of ara-C and cytidine on four phases. On the low-polarity phases, these derivatives were poorly separated from each other necessitating MS monitoring of the extent of the methylation reaction. The dimethyl-TMS derivatives of ara-C and cytidine could not be separated from each other on SE-30 or OV-210 but reasonably good separation was achieved on the other two phases. Of these, OV-17 produced the sharpest peaks. Retention times of both derivatives increased with the polarity of the phases in an analogous manner to those reported by Hattox and McCloskey[3] and Miller et al.[5] for TMS derivatives.

For single-ion monitoring, the dimethyl-TMS derivative was better than either the monomethyl-TMS or the methylacetate derivative as its spectrum contained an abundant molecular ion at m/e 487 (34%). Table II shows a comparison of the MS properties of all the derivatives and Fig. 6 shows the mass spectrum of the dimethyl-TMS derivative of ara-C. The major fragment ions in the spectrum of the dimethyl-TMS derivatives of both nucleosides reflected fragmentations typical of those previously reported for nucleoside-TMS[17,18] and related derivatives[17,19]. The base peak [m/e 217, (TMSO)$_2$C$_3$H$_3$] and the abundant ion at m/e 103 (CH$_2$O$^+$=TMS) originated from the pentose moiety and represent typical sugar-TMS fragment ions[20]; compositions of the ions were checked by their shifts in the spectra of the [^{2}H$_9$]TMS derivatives[21]. Other sugar-derived ions included the series [M — (base + H)]$^+$ (m/e
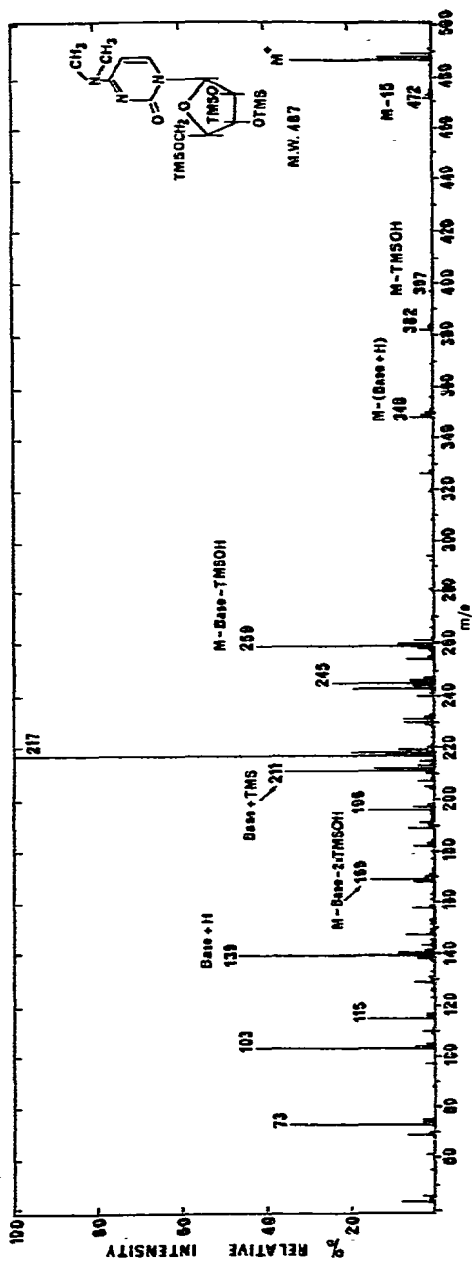
Fig. 6. 25-eV mass spectrum of the dimethyl-TMS derivative of ara-C.

TABLE II

MASS SPECTRAL DATA FOR THE DERIVATIVES OF ara-C AND CYTIDINE

m/e values; relative abundance in parentheses.

Compound	Derivative	M^+	$[M-R]^{+*}$	Base	Other abundant ions (see text)					
Ara-C	Me-Ac	425	410	139	259	97	43	152	157	
		(3)	(16)	(100)	(53)	(35)	(32)	(14)	(14)	
	Me-TMS	473	458	217	259	103	197	169		
		(4)	(2)	(100)	(49)	(30)	(25)	(15)		
	Me$_2$-TMS	487	472	217	139	259	103	211	245	243
		(34)	(2)	(100)	(46)	(43)	(43)	(36)	(25)	(19)
	MO-TMS	489	474	259	213	103	245	217	243	348
		(25)	(3)	(100)	(91)	(68)	(60)	(47)	(38)	(26)
	EO-TMS	503	488	259	227	245	103	217	243	348
		(29)	(3)	(100)	(94)	(87)	(83)	(53)	(49)	(27)
	BuO-TMS	531	516	259	245	103	255	217	243	258	348
		(28)	(3)	(100)	(98)	(81)	(79)	(59)	(49)	(42)	(28)
	ADMS	565	524	196	173	101	149	129	408	182
		(9)	(56)	(100)	(46)	(42)	(37)	(34)	(34)	(31)
Cytidine	Me-Ac	425	410	139	259	97	43	157	152	
		(2)	(6)	(100)	(69)	(38)	(34)	(16)	(14)	
	Me$_2$-TMS	487	472	217	103	259	139	211	245	243
		(23)	(7)	(100)	(59)	(56)	(34)	(34)	(29)	(23)
	MO-TMS	489	474	259	103	245	217	243	169	115	348
		(21)	(6)	(100)	(70)	(43)	(33)	(30)	(21)	(21)	(17)
	EO-TMS	503	488	259	103	227	245	217	243	169	348
		(25)	(18)	(100)	(74)	(68)	(58)	(35)	(33)	(23)	(19)
	BuO-TMS	531	516	259	103	245	255	217	243	348	183
		(26)	(8)	(100)	(79)	(74)	(51)	(44)	(39)	(21)	(19)

* R = CH$_3$ in all derivatives except the ADMS derivative where R = CH$_2$-CH=CH$_2$.

348), (M — base — TMSOH)$^+$ (m/e 259), [M — base — (2 × TMSOH)]$^+$ (m/e 169) and m/e 245, [M — (base + H) — CH$_2$OTMS]$^+$ (ref. 18). Ions containing the base were observed at m/e 139 (base + H) and m/e 211 (base + TMS) in the spectra of the dimethyl-TMS derivatives and at 14 a.m.u. lower in the monomethyl TMS spectrum. There was little difference between the spectra of the ara-C and cytidine derivatives and the ions involving migration of two hydrogens to the base, abundant in compounds containing free hydroxyl groups[19], were absent.

Detection limits achieved during single-ion monitoring of the molecular ion from the dimethyl-TMS derivative were much better than could be achieved with the methylacetate derivative as can be seen from Fig. 7 which shows a comparison of the concentration–response curves of four derivatives of ara-C. A detection limit of 50 pg was achieved with a 3:1 signal-to-noise ratio on 3% OV-17 at 265° with a linear response up to at least 10 ng.

In an attempt to increase the sensitivity still further, substituted silyl derivatives such as the tert.-butyldimethylsilyl ethers (TBDMS) which are known to produce abundant (M — tert.-butyl)$^+$ ions[22] were investigated. However, the fully silylated TBDMS derivatives could not be prepared, presumably because of the bulky nature of the TBDMS group[22-25], and as the use of a three step reaction involving TMS ether formation of the unprotected hydroxyl groups[24] was undesirable for pharmacokinetic work, this derivative was not pursued further.
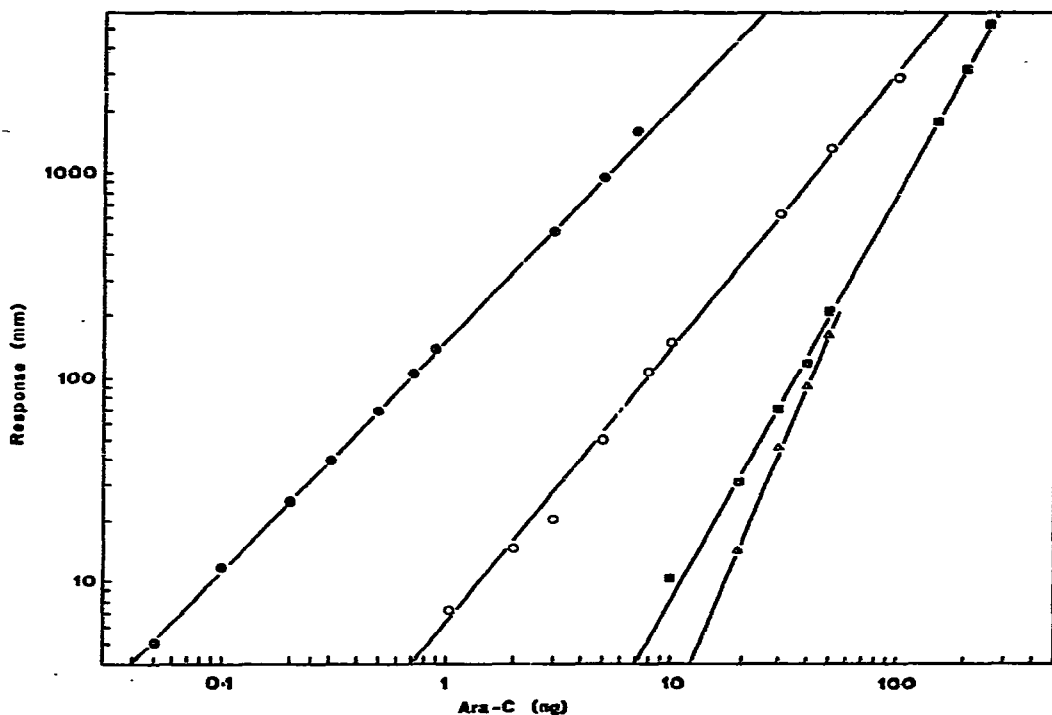
Fig. 7. Comparison of GC–MS responses of derivatives of ara-C using single-ion monitoring. The response axis has been corrected for the amplifier setting. ● = Me$_2$-TMS; ○ = Me-Ac; ■ = MO-TMS; △ = BuO-TMS.

Allyldimethylsilyl (ADMS) ethers[26,27], which fragment in a similar way to the TBDMS ethers and produce abundant (M − allyl)$^+$ ions, are much less bulky and it was found possible to prepare the dimethyl-ADMS derivatives nearly as readily as the methyl-TMS ethers. These derivatives gave very abundant (M − 41)$^+$ ions (m/e 524, 56%) but were not pursued further because of difficulty in obtaining a single peak. Propyldimethylsilyl derivatives[28–30] were also investigated but the (M − Pr)$^+$ ions were rather weak and these derivatives were not pursued further.

As an alternative, the formation of alkyloximes by the reaction of the nucleoside with alkyloxyamine hydrochlorides[12] was also investigated. Oxime formation was achieved by heating the nucleoside with either methoxy, ethoxy or butoxyamine hydrochloride in pyridine for 4 h at 80° and the product was converted into its TMS ether as before. Single GLC peaks were obtained for all three oximes of both ara-C and cytidine on the four phases with no evidence of the formation or separation of *syn* and *anti* isomers. The derivatives of ara-C and cytidine separated on all phases except OV-210 with the ara-C derivatives eluting first in all cases (Table I). All the derivatives produced reasonably abundant molecular ions and fragment ions similar to those observed in the spectra of the methyl-TMS derivatives (Table II). Substituent labelling of the oxime alkyl group provided a convenient method for the identification of ions containing the derivatized base; these were predominantly the (base + H)$^+$ and (base + TMS)$^+$ ions. The spectra differed from those of the methyl-TMS

derivatives in that the ion at m/e 259 [(M − base − TMSOH)$^+$] rather than m/e 217 was the base peak. Again the spectra of the derivatives of ara-C and cytidine were similar although the base peaks carried a larger percentage of the total ion current in the spectra of the cytidine derivatives.

Unfortunately, although these derivatives were readily prepared and gave abundant molecular ions, the sensitivities achieved by single-ion monitoring were poor as can be seen from Fig. 7. The detection limits were around 5 ng and the signal-to-noise ratio was also poor because of coincidence with abundant ions from column bleed, particularly with the ethyl (m/e 503) and butyl oximes (m/e 531). Consequently these derivatives were not investigated further.

CONCLUSIONS

Although the dimethyl-TMS derivative of ara-C gave the best GC–MS sensitivity, the acetyl methyl derivative proved to be the most satisfactory derivative for studies of ara-C plasma concentrations; the oximes were rejected on grounds of low sensitivity. Attempts to prepare the dimethyl-TMS derivative, although successful with the pure drug, gave inconsistent results in the presence of plasma extract with the production of several GLC peaks. A method has not yet been found to overcome this difficulty. On the other hand, the acetyl methyl derivative gave reproducible results and, in addition, its stability towards water enabled further purification to be carried out after derivatization of the plasma extract. Also the readily prepared [^{2}H$_{12}$]acetyl methyl derivative can be used as carrier and as a standard. Thus, using this derivative, a method for the extraction and analysis of ara-C from plasma and the determination of the pharmacokinetic profile of this drug in leukaemic patients by GLC and GC–MS has been developed[16].

ACKNOWLEDGEMENT

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