^{18}O-LABELED NUCLEOSIDES 3. PREPARATION AND MASS SPECTROMETRIC EVALUATION OF $^{18}O2$-LABELED ARA-C AND ARA-U†. (1)

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SUMMARY

A facile method for the preparation of ^{18}O labeled ara-C and ara-U is described. The method provides a means of incorporating ^{18}O specifically at the 2-position of the nucleobase. The site and level of label was established using mass spectrometry.

Key Words: Nucleosides, Stable Isotopes, Mass Spectrometry

INTRODUCTION

Cyclo-C and ara-C are widely used clinically in the treatment of various types of leukemia and related haematological disorders (2-4). Even though it has been shown that ara-C undergoes rapid enzymatic deamination to give ara-U, the precise mode of action of ara-C is still not fully understood (5-6). The in vivo activation and deactivation of cyclo-C has been shown to proceed by ring-opening to give the active anabolite ara-C, which is subsequently deaminated to ara-U (7). The kinetics of these transformations may be of importance in designing more efficacious dosage schedules in the clinical treatment of neoplasms.

Among a number of published techniques examining the pharmacokinetics of ara-C (8), mass spectrometry was shown to be of potential value. Metabolic interconversions of ara-C were studied utilizing the permethyl derivative of

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Abbreviations: †Ara-C, 1-(β-D-arabinofuranosyl)cytosine; ara-U, 1-(β-D-arabinofuranosyl)uracil; cyclo-C, O^2,2'-anhydro-1(β-D-arabinofuranosyl)cytosine; cyclo-U, O^2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil.

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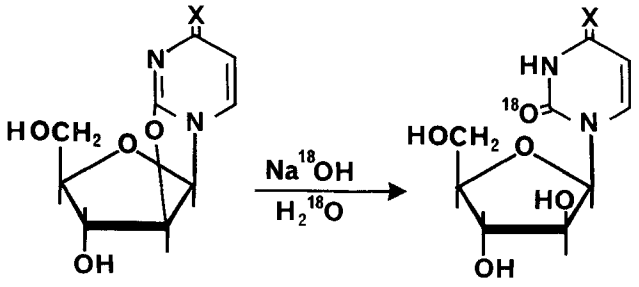
ara-C (9), as well as using the deuterated acetyl-methyl derivatives (10). Alternatively, ara-C and ara-U, transformed to their volatile trimethylsilyl (TMS) derivatives, could readily be separated and characterized according to well-established GC/MS procedures (11). Thus, the presence of a properly situated stable isotope label such as ^{18}O in the ara-C molecule would allow the quantitative determination of the levels of both ara-C and ara-U. Endogenous cytidine and uridine would not interfere with these determinations because the ara-analogues elute as separate peaks from the ribo-isomers when employing the appropriate gas chromatographic conditions (12).

Previous reports on the chemistry of $O^2,2'$-anhydro nucleosides have shown the anhydro bridge to be readily cleaved by sodium hydroxide to produce the ring-opened arabinoside (13). Based on previous work in this laboratory (14), and applying the above-mentioned reaction conditions, we have developed a simple method for the preparation of specifically labeled ara-C and ara-U.

RESULTS AND DISCUSSION

The treatment of cyclo-C and cyclo-U with $Na^{18}OH$ resulted in the formation of labeled ara-C (2) and ara-U (4), as illustrated in Scheme I.

Scheme I: Ring-opening of the $O^2,2'$-anhydro bridge in ara-C and ara-U



1: X = NH·HCl 2: X = NH

3: X = O 4: X = O

The mass spectra of the labeled arabinosides 2 and 4 are identical to the spectra of the unlabeled molecules except for the m/z + 2 shift of a number of specific ions. The spectrum of 2 shows a shift of the molecular ion from m/z 243 to m/z 245 and from m/z 244 to m/z 246 in the spectrum of arabinoside 4 indicating the incorporation of one ^{18}O atom. The prominent ions associated with the base (B, B+H, B+2H, B+30) (11) are also shifted by +2 mass units, while the peak for the sugar ion remains at m/z 133. These results show that during nucleophilic opening of the $O^2,2'$-bridge ^{18}O is incorporated specifically at the 2-position of the pyrimidine base. Several characteristic ions of ara-C and ara-U are compared to their labeled counterparts in Table I.

Table I: Characteristic ions of labeled and unlabeled ara-C and ara-U.

Ion	ara-C	2	ara-U	4
	m/z (RI)[a]	m/z (RI)	m/z (RI)	m/z (RI)
M^+	243 (3)	245 (2)	244 (5)	246 (2)
B[b]	111 (52)	113 (50)	112 (33)	114 (18)
B + H	112 (100)	114 (100)	113 (100)	115 (100)
B + 2H	113 (8)	115 (14)	114 (11)	116 (9)
B + 30	140 (27)	142 (30)	141 (19)	143 (10)
B + 41	151 (43)	153 (54)	-	-
S[b]	133 (1.2)	133 (2)	133 (43)	133 (20)

a: RI = relative intensity, b: B = base, S = sugar

The purified arabinosides 2 and 4 were converted to their TMS-derivatives and their mass spectrometric fragmentation patterns agree with those of the non-labeled analogues except for the expected +2 shift in several characteristic ions. The TMS-derivative of 2 does not show a molecular ion peak at m/z 533. However, the M-15 peak at m/z 518 indicates the incorporation of one ^{18}O. The TMS-derivative of 4 shows the molecular ion at m/z 534 and the M-15 at m/z

519, which again confirms ^{18}O-incorporation in 4.

The level of isotope incorporation was obtained by slowly scanning (25 sec/decade) the mass range from 500-550 amu and averaging the aquired spectra to eliminate bias. Percent isotope incorporation was calculated using the M-15 ion region as reference. Relative intensities, shown in Table II, indicate the presence of 96.5% labeled ara-C (2), and 97.5% labeled ara-U (4).

Table II: M-15 ion region of TMS-derivatives

	Relative Intensity			
(m/z)	ara-C	2	ara-U	4
515	-	-	-	-
516	100	3.5	-	-
517	68	1	100	2.5
518	34	100	43	1
519	16	45	23	100
520	6	24	7	45
521	2	7	1.5	26
522	-	3	-	8
523	-	-	-	3
524	-	-	-	-

The site of ^{18}O-incorporation was confirmed in the TMS-derivatives of 2 and 4 by the +2 shift of all the major fragments associated with the base, such as B+H+TMS and no shift being observed in the sugar fragment ions such as S-H+3TMS, $C_3H_3O_2$+2TMS, and 5'-CH_2O+TMS. Several characteristic ions in the spectra of tetrasilylated 2 and 4 are listed in Table III, along with the corresponding peaks of the unlabeled analogues.

Table III. Selected ions in the mass spectra of persilylated nucleosides*.

Ion	ara-C(TMS)	2(TMS)	ara-U(TMS)	4(TMS)
	m/z (RI)	m/z (RI)	m/z (RI)	m/z (RI)
M+	531 (1)	-	532 (0.3)	534 (0.5)
M-CH_3	516 (10)	518 (8)	517 (4.7)	519 (4.4)
S+3TMS	349 (2)	-	349 (2)	349 (2)
S+3TMS-H	348 (1)	348 (6)	348(2)	348 (1.8)
B + H + TMS	256 (25)	258 (48)	257 (10)	259 (28)
C_3H_3 $(OTMS)_2$	217 (60)	217 (61)	217 (47.5)	217 (61)
5'-CH_2OTMS	103 (7.5)	103 (7)	103 (8.5)	103 (14)

* Spectral assignments are based on published work (11).

The TMS-derivatives of 2 and 4 can also be prepared *in situ* by treating the neutralized and dried hydrolysis reaction mixture with the silylating reagent. Gas chromatographic analysis of the silylated mixture affords spectra identical to the spectra of compounds purified prior to silylation, and obtained by direct insertion probe.

In summary, the described method offers a simple means of preparing internal standards highly labeled with ^{18}O in the O^2 position of the base in ara-C and ara-U. We believe these compounds will be of value in studying the metabolism and the distribution of cyclo-C and ara-C by gas chromatography/mass spectrometry (GC/MS).

EXPERIMENTAL

Mass spectra were recorded on a Varian MAT 311A mass spectrometer interfaced to a Varian 3700 gas chromatograph (2m x 2mm, 3% OV-17 on chromsorb w) via an all glass jet separater at 290°C. The column oven temperature was programmed at 4°C/min from 160°-260°C; the mass spectrometer was operated with

ion source temperature of 250°C and ionizing electron energy of 70eV. Preparative thin layer chromatography (TLC) was performed on plates 20x20 cm coated with silica gel GF, 2000 microns thickness, (Analtech, Newark, Delaware).

Cyclo-C and cyclo-U were purchased from Sigma Chem. Co., St. Louis, Mo; $H_2{}^{18}O$ (99% ^{18}O) was obtained from Norsk Hydro Sales, New York, N.Y. 1N $Na^{18}OH$ was prepared under nitrogen atmosphere by cautiously adding sodium metal (40 mg, 1 mmol) to 1 ml $H_2{}^{18}O$; TMS-derivatives were prepared using N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) according to reported procedures (12).

1-(β-D-Arabinofuranosyl)cytosine-[O^2-^{18}O] (2): O^2,2'-Anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (1) (14 mg, 0.05 mmol) was suspended in 60 μL 1N $Na^{18}OH$ (99% ^{18}O) and the temperature was maintained at 35°C for 24 hours. From the clear solution 2 was isolated on silica gel by preparative TLC, R_f 0.20, with ethyl acetate/n-propanol/water (4:1:2) (upper layer) as the mobile phase. The band containing 2 was scraped from the plate and eluted with methanol. The solvant was removed under reduced pressure to give 2 as a semicrystalline product in 55% yield. Treatment with 1N HCl, evaporation of the aqueous phase, and recrystallization from ethanol afforded 2 as the hydrochloride with m.p. 188°C (decomp.) lit. 191-194°C (decomp.) (15)

1-(β-D-Arabino-furanosyl)uracil [O^2-^{18}O] (4): O^2,2' -Anhydro-1-(β-D-arabinofuranosyl)uracil (3) (25 mg, 0.1 mmol) was dissolved in 50 μL 0.3N $Na^{18}OH$ (99% ^{18}O). After standing at 35°C for 24 hours labeled ara-U (4) was isolated in 81% yield by preparative TLC, R_f 0.74, as previously described for compound 2; m.p. 217-222°C (ethanol) lit. 222-224°C (16).

1- (β-D-Arabinofuranosyl)cytosine and 1-(β-D-arabinofuranosyl)uracil were prepared analogously by treating cyclo-C and cyclo-U with $Na^{16}OH$, followed by purification procedures described for 2 and 4. The mass spectra of the obtained arabinosides were identical to published spectra of ara-C and ara-U (17).

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