Journal of Chromatography A, 657 (1993) 395–404
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 519

Synthesis of novel tellurium containing analogues of choline and acetylcholine and their quantitation by pyrolysis–gas chromatography–mass spectrometry

S.E. Harris

Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208 (USA)

L.A. Silks, III

Los Alamos National Laboratory, Biochemistry and Spectroscopy Section, INC-4: MS C346, Los Alamos, NM 87545 (USA)

R.B. Dunlap and J.D. Odom

Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208 (USA)

J.W. Kosh*

College of Pharmacy, University of South Carolina, Columbia, SC 29208 (USA)

(First received March 9th, 1993; revised manuscript received August 12th, 1993)

ABSTRACT

Methods for the synthesis and quantitation of the novel choline analogues, telluronium choline and acetyltelluronium choline, are described. An assay procedure utilizing pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) with cold trapping was developed with [$^2H_4$]telluronium choline and [$^2H_4$]acetyltelluronium choline as internal standards. The telluronium compounds were ion-pair extracted from tissue with dipicrylamine, washed with 2-butanone, and pyrolyzed prior to GC–MS analysis. The compounds were monitored using selected ion monitoring at *m/z* 232 and *m/z* 190 for acetyltelluronium and telluronium choline, respectively, and at *m/z* 236 and *m/z* 194 for the analogous deuterated internal standards. The assay was linear over a range of 20 pmol–20 nmol of compound taken through the assay.

INTRODUCTION

A number of analogues of choline [$(CH_3)_3N^+CH_2CH_2OH$] and acetylcholine [$(CH_3)_3N^+CH_2CH_2OC(O)CH_3$] have been utilized to study cholinergic neurotransmission. Many of these analogues have subsequently been shown to qualify as false cholinergic neurotransmitters [1–5] and to prevent acetylcholinesterase inhibitor toxicity [6,7].

In general, alteration of the alkyl or quaternary region of the choline molecule has produced the most active choline analogues. Analogues with modified alkyl regions include homocholine, β-methylcholine [8], monoethylcholine, diethylcholine, triethylcholine and pyrolidinium choline [5]. Modifications of the quaternary nitrogen include replacement of the nitrogen with another group 15 atom to yield

* Corresponding author.

0021-9673/93/$06.00

phosphocholine and arsenocholine [1] and stibocholine [2]. Replacements of the quaternary nitrogen that result in a quaternary neutral structure include carbocholine and silicocholine [9], whereas replacement of the nitrogen atom by a group 16 atom results in tertiary, positively charged structures including sulfocholine [3] and, most recently, selenonium choline [10].

Data from the recent characterization of the biological activity of selenonium choline [$(CH_3)_2Se^+CH_2CH_2OH$, TeCh] demonstrate that the compound satisfies many of the criteria of a false neurotransmitter [7,11]. It was therefore of interest to examine the tellurium-containing analogue, telluronium choline [$(CH_3)_2Te^+CH_2CH_2OH$]. Because of the structural similarity to selenonium choline, telluronium choline should be expected to behave biologically in a similar manner. The fact that tellurium occurs naturally in the earth's crust and is found in high concentrations in selected foods [12], suggests the possibility of its incorporation into biological compounds. Although tellurium-containing amino acids have not been detected in mammalian tissue, partial incorporation of telluromethionine in place of methionine in dihydrofolate reductase has been demonstrated in *Escherichia coli* [13]. Because the possibility exists that telluronium choline might occur in nature and may act as a cholinergic false transmitter, it was of interest to undertake its synthesis and to develop a method for its quantitation. The present work therefore describes the synthesis of the novel trigonal choline analogues telluronium choline and acetyltelluronium choline [$(CH_3)_2Te^+CH_2CH_2OC(O)CH_3$, ATeCh] and their quantitation using a pyrolysis–gas chromatography–mass spectrometric assay.

EXPERIMENTAL

Spectroscopic studies

NMR spectra were recorded as C^2HCl_3 or ^2H_2O solutions, unless otherwise noted, on either a Bruker AM 500, AM 300, WP-300 or AM-200 spectrometer. The resonance frequencies for ^1H, ^{13}C and ^{125}Te are 300.133, 75.427 and 94.692 MHz, respectively. Proton spectra were the result of 32 transients with a 64K data table and were referenced externally to tetramethylsilane (TMS) or as values in parts per million relative to $CHCl_3$. ^{13}C spectra were obtained with proton decoupling and were the result of 128–256 transients with a 64K data table and were either referenced to external TMS or with respect to internal C^2HCl_3 (δ-77.0). The ^{125}Te spectra were performed with proton decoupling and were the result of 1024 or 2048 transients with a 64K data table and the chemical shifts are reported as values in parts per million relative to $Te(OH)_6$ [14,15]. The concentration of ^{125}Te NMR samples was approximately 0.2 *M*. Spectra were run with a 55 kHz sweep width using a 2.8 μs tip angle (90° tip angle is 7 μs) and a 0.5 s relaxation delay. The probe was maintained at ambient temperature. All spectra were taken using 5 mm NMR tubes.

Accurate mass spectra were measured on a VG 70SQ GC–MS spectrometer using glycerin ($m/z = 185$) as a reference (Fig. 1). The exact masses for TeCh and ATeCh were 199.9853 and 241.9963, respectively, while the calculated mass for $C_4H_{11}O^{125}Te$ was 199.9854 (0.5 ppm error) and the mass for $C_6H_{13}O_2{}^{125}Te$ was 241.9960 (1.2 ppm error). Elemental analyses were performed at the Los Alamos National Laboratory and at Atlantic Microlabs. (Norcross, GA, USA).

MS analysis of the telluronium compounds was accomplished using the pyrolysis GC–MS method described by Terry *et al.* [10], with the following changes: pyrolysis temperature was 290°C, interface temperature was 190°C, and pyrolysis products were cold trapped for 6 min with an injector purge time of 6 min. The prepared samples were analyzed using a Hewlett-Packard gas chromatograph (5890)–mass spectrometer (5970) and a Stabilwax column (Restek, 30 m × 0.25 mm I.D., 0.5 μm phase). Selected ion monitoring (SIM) was employed for compound detection using m/z 232 ($[CH_3–Te–CH_2–CH_2–O–C(O)CH_3]^+$) and 236 ($[CH_3–Te–C^2H_2–C^2H_2–O–C(O)CH_3]^+$), which corresponded to the base peaks for the thermally demethylated acetyltelluronium choline (d_0) and [$^2H_4$]acetyltelluronium choline (d_4) variants, re-

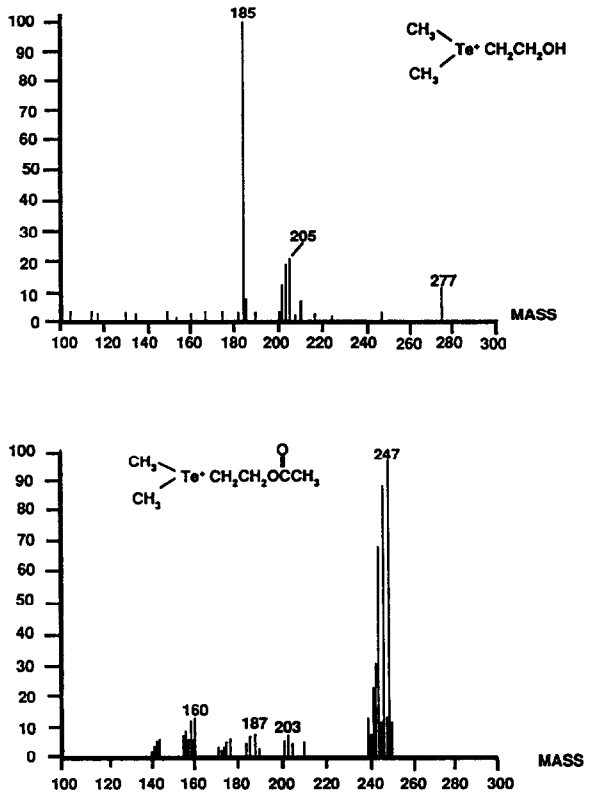

Fig. 1. Fast atom bombardment spectra (positive ion) of (top) d_0-telluronium choline$_0$- and (bottom) d_0-acetyltelluronium choline$_0$-. Spectra were obtained following a standard static procedure using a VG 705Q mass spectrometer and data system.

spectively. Similarly, d_0- and d_4-telluronium choline compounds were monitored at m/z 190 ($[CH_3–Te–CH_2–CH_2–O–H]^+$) and m/z 194 ($[CH_3–Te–C^2H_2–C^2H_2–O–H]^+$), respectively, corresponding to the base peaks of the demethylated variants. Since the Autotune function optimizes the instrument for perfluorotributylamine (PFTBA), a shift of ± 0.1 m/z is frequently observed for the ion of interest. Therefore, data collection for the telluronium compounds consisted of a three-ion bracket surrounding m/z 232 (231.9, 232.0, 232.1), m/z 236 (235.9, 236.0, 236.1), m/z 190 (189.9, 190.0, 190.1) and m/z 194 (193.9, 194.0, 194.1).

Because the ion abundances for the telluronium compounds were typically low, the areas for the ions in a three-ion bracket were summed together and used for quantitation purposes. The areas were then corrected for area spillover using a matrix based Pascal program written by one of the authors (J.W.K.). The area correction algorithm used was essentially the same as that reported by Jenden and Silverman [16] for correcting spillover between deuterated and non-deuterated variants of choline and acetylcholine. The extent of spillover for each isotope was determined using a set of three "matrix" tubes containing only d_0- or d_4-, or a mixture of both variants for each set of 12–16 experimental tubes (See Table I).

TABLE I

REPRESENTATIVE NORMALIZED AREA CONTRIBUTIONS BETWEEN IONS OF INTEREST

Tubes containing only d_0-, or only d_4-, or a mixture of d_0- and d_4-telluronium choline and acetyltelluronium choline were processed according to the assay protocol and subjected to pyrolysis–GC–MS analysis. The areas obtained during selected ion monitoring analysis for m/z 190, 194, 232 and 236 were normalized by dividing each area by the largest value obtained for that sample. The normalized areas were subsequently used in matrix calculations to provide corrected area values for the experimental samples.

	Acetyltelluronium choline, m/z		Telluronium choline, m/z	
	232	236	190	194
d_0	1.0	0.02098	1.0	0.01791
d_4	0.53969	1.0	0.54647	1.0
$d_0 + d_4$	1.0	0.74231	1.0	0.67469

Synthetic reagents

Elemental tellurium was obtained from Alfa Products as a gray powder and used without further purification. Chloroethanol was obtained from Aldrich, distilled, and stored over 4 Å molecular sieves before use. Methyl trifluoromethanesulfonate, iodomethane and ethereal solutions of methyllithium were obtained from Aldrich, and used without further purification. 2-Bromo[1-$^2H_2$,2-$^2H_2$]ethanol was obtained from Cambridge Isotopes and used without further purification. The concentration of methyllithium reagents in commercial solutions was determined by titration of diphenylacetic acid to the yellow end point [17]. Tetrahydrofuran (THF) was distilled from sodium/benzophenone prior to

Preparation of 2-(methyltellurenyl)ethanol (1)

In a flame dried and purged (nitrogen) 250-ml three-necked flask, fitted with a septum, gas inlet, ground glass stopper and magnetic stirring bar, were added 2.00 g of elemental tellurium (15.7 mmol), and 50 ml of dry THF. To the stirring tellurium–THF suspension, under nitrogen, were added dropwise, at 0°C, 11.3 ml (1.4 *M*) methyllithium. As the addition reached the end-point (1 equivalent) the dark brown suspension lightened to give a clear yellow solution. Stirring was continued for 5 min, and subsequently the temperature was lowered to −78°C. Then 1.16 ml of 2-chloroethanol (17.2 mmol) were added dropwise. Stirring was continued for 1 h at −78°C, then the mixture was allowed to warm slowly to room temperature and stirring was continued for 12 h. The reaction mixture was then filtered through a pad of SiO_2 (EM Science; 230–400 mesh; 40–200 μm) and all volatiles were removed *in vacuo*. The crude material was then subjected to flash column chromatography (SiO_2, 230–400 mesh) using $CHCl_3$ as the eluent. This gave 2.94 g (76%). NMR (in ppm): ^1H (C^2HCl_3), δ 1.87 [s, J (^1H–^{125}Te) = 20.8 Hz, 3H, CH_3Te], 2.1 (broad s, 1H, CH_2OH), 2.75 (t, J = 6.8 Hz, $TeCH_2$), 3.75 (m, 2H, CH_2OH).

Preparation of 2-(methyltellurenyl)ethyl acetate (2)

In a flame dried and purged (nitrogen) 250-ml three-necked flask, fitted with a septum, gas inlet, ground glass stopper and magnetic stirring bar, were added 2.00 g of elemental tellurium (15.7 mmol), and 50 ml of dry THF. To the stirring tellurium–THF suspension, under nitrogen, were added dropwise, at 0°C, 11.1 ml (1.4 *M*) methyllithium. As the addition reached the end-point (1 equivalent) the dark brown suspension lightened to give a clear yellow solution. Stirring was continued for 5 min, and subsequently the temperature was lowered to −78°C. Then 1.16 ml of 2-chloroethanol (17.2 mmol) were added dropwise. Stirring was continued for 1 h at −78°C then the mixture was allowed to warm slowly to room temperature and stirring was continued for 12 h. Then 2.20 ml (79 mmol) of triethylamine were added dropwise at 0°C. This was followed by the addition of 1.5 ml (79 mmol) of acetic anhydride. After 12 h the reaction was determined to be incomplete (TLC) and an additional 5 ml of acetic anhydride was added. Stirring was continued until consumption of **1** was complete as determined by TLC analysis. The reaction mixture was concentrated under reduced pressure and then chromatographed using a $CHCl_3$–heptane mixture. This gave 2.5 g (70%).

Alternatively, treatment of 0.41 g (2.18 mmol) 2-(methyltellurenyl)ethanol with 0.21 ml (2.3 mmol) of acetic anhydride, 0.185 ml (2.3 mmol) of pyridine, in 20 ml of dichloromethane for 12 h afforded a 78% yield of **2**. Data for **2**: NMR (in ppm): ^1H (C^2HCl_3), δ 1.87 (s, 3H, CH_3Te), 1.97 (s, 3H, CH_3CO), 2.70 (t, J = 7.8 Hz, 2H, $TeCH_2$), 4.25 (t, J = 7.8 Hz, 2H, CH_2OAc); ^{13}C, −22 [J (^{13}C–^{125}Te) = 157 Hz, CH_3Te], 0.9 [J (^{13}C–^{125}Te) = 166 Hz, $TeCH_2$], 20.6 (CH_3CO), 65.9 (CH_2OAc), 169.9 ($COCH_3$).

Preparation of 2-(dimethyltelluronium)-ethyl acetate trifluoromethyl sulfonate, acetyltelluronium choline (3)

To 2.5 g (10.9 mmol) of **2** in 5 ml of methylene chloride, chilled to 0°C was added 1.23 ml of methyl trifluoromethyl sulfonate (10.8 mmol) dropwise, under nitrogen. The mixture was then warmed to ambient temperature and stirred until all starting material was consumed as shown by TLC. The solution was then washed with diethyl ether. The resulting oil was then dried *in vacuo*

to give 4.29 g (97%). NMR (in ppm): ^{1}H ($^{2}H_2O$), δ 2.07 (s, 3H, CH_3CO), 2.35 [2, J (^{1}H–^{125}Te) = 25 Hz], 6H, $(CH_3)_2Te$], 3.16 (t, J = 6.0 Hz, 2H, $TeCH_2$), 4.50 (t, J = 6.0 Hz, 2H, CH_2OAc); ^{13}C, 5.0 [J (^{13}C–^{125}Te) = 152 Hz, $(CH_3)_2Te$], 21.6 (CH_3CO), 24.6 [J (^{13}C–^{125}Te) = 150 Hz, $TeCH_2$], 62.3 (CH_2OAc), 121.4 [q, H (^{13}C–^{19}F) = 318.4 Hz, $CF_3SO_3^-$], 174.5 ($COCH_3$); ^{125}Te, 493.0 (0.24 *M*). Accurate mass by positive ion fast atom bombardment gave 241.9963 (calculated mass 241.9960) with an error of 1.2 ppm. Elemental analysis calculated for $C_7H_{13}O_5F_3STe^+$: C, 21.35; H, 3.33. Found: C, 20.86; H 3.40.

Preparation of 2-(dimethyltelluronium)-ethanol iodide, telluronium choline (4)

Three ml of iodomethane (48.2 mmol) were added neat to 2.23 g of **1** (11.9 mmol) which was being rapidly stirred at 0°C. The reaction was mildly exothermic. The mixture was stirred until TLC analysis indicated **1** was consumed. The residue was taken up in water and washed with methylene chloride until the aqueous solution was clear and colorless. The solution was then dried *in vacuo* to give 3.74 g of a cream colored solid (96%). NMR (in ppm): ^{1}H ($^{2}H_2O$), δ 2.35 [s, J (^{1}H–^{125}Te) = 24 Hz, 6H, $(CH_3)_2Te$], 3.28 (t, J = 6.3 Hz, 2H, $TeCH_2$), 4.03 (m, 2H, CH_2OH); ^{13}C, 6.3 [J (^{13}C–^{125}Te) = 148 Hz, $(CH_3)_2Te$], 32.7 [J (^{13}C–^{125}Te) = 168 Hz, $TeCH_2$], 60.2 (CH_2OH); ^{125}Te, 448.1 (0.18 *M*). Accurate mass by positive ion fast atom bombardment gave 199.9853 (calculated mass 199.9854) with an error of 0.5 ppm. Elemental analysis calculated for $C_4H_{11}IOTe^+$: 14.79; H, 3.58. Found: C, 14.57; H, 3.36.

Preparation of 2-(methyltellurenyl)-[1-$^{2}H_2$,2-$^{2}H_2$]ethanol (5)

The synthesis procedure used was identical to that described for **1**, except that 11.6 ml (1.0 *M*) of methyllithium were added to 1.48 g of elemental tellurium (11.6 mmol) followed by 1.5 g of 2-bromo[1-$^{2}H_2$,2-$^{2}H_2$]ethanol (11.6 mmol). The final eluent for chromatography was $CHCl_3$–diethyl ether (95:5). Final yield was 1.65 g (74%). NMR (in ppm): ^{1}H ($C^{2}HCl_3$), δ 1.85 [s, J (^{1}H–^{125}Te) = 20.8 Hz, 3H, CH_3Te], 2.6 (broad s, 1H, CH_2OH); ^{13}C, −22 $(CH_3)Te$, 7 (m, $C^{2}H_2Te$), 63 (m, $C^{2}H_2OH$).

Preparation of 2-(methyltellurenyl)-[1-$^{2}H_2$,2-$^{2}H_2$]ethyl acetate (6)

The synthesis procedure used was identical to that described for **2**, except that 11.6 ml of methyllithium (1.0 *M*) were added to 1.48 g of elemental tellurium (11.6 mmol) followed by 1.5 g of 2-bromo[1-$^{2}H_2$,2-$^{2}H_2$]ethanol. Then 2.81 ml (36.3 mmol) of dry pyridine was added dropwise at 0°C followed by the addition of 3.28 ml (29.7 mmol) of acetic anhydride. Final yield was 1.5 g (55%). NMR (in ppm): ^{1}H ($C^{2}HCl_3$), δ 1.93 (s, 3H, CH_3Te), 2.04 (s, 3H, CH_3CO); ^{13}C, −22 (CH_3Te), 21 (CH_3CO), 170.6 ($COCH_3$), the deuterated carbons were not observed.

Preparation of 2-(dimethyltelluronium)-[1-$^{2}H_2$,2-$^{2}H_2$]ethanol iodide, d_4-telluronium choline (7)

The synthesis procedure used was identical to that described for **4**, except that 1.45 g of **5** (7.6 mmol) was used. The procedure yielded 1.53 g of a cream colored solid (60%). NMR (in ppm: ^{1}H ($^{2}H_2O$), 2.28 [s, J (^{1}H–^{125}Te) = 24 Hz, 6H, $(CH_3)_2Te$]; ^{13}C, 6.3 [J (^{13}C–^{125}Te) = 149 Hz, $(CH_3)_2Te$], 32.2 (m, $TeC^{2}H_2$), 59.7 ($C^{2}H_2OH$).

Preparation of 2-(dimethyltelluronium)-[1-$^{2}H_2$,2-$^{2}H_2$]ethyl acetate trifluoromethyl sulfonate, d_4-acetyltelluronium choline (8)

The synthesis procedure used was identical to that described for **3**, except that 0.73 ml of methyl trifluoromethyl sulfonate (6.5 mmol) were added to 1.5 g of **6** (6.3 mmol). Water (10 ml) was then added and the final aqueous solution was then dried *in vacuo* (the temperature was not allowed to rise above 15°C) to give 1.57 g (63%). NMR (in ppm): ^{1}H ($^{2}H_2O$), δ 1.93 (s, 3H, CH_3CO), 2.21 [s, J (^{1}H–^{125}Te) = 24 Hz, 6H, $(CH_3)_2Te$]; ^{13}C, 5.4 [J (^{13}C–^{125}Te) = 152 Hz, $(CH_3)_2Te$], 21.8 (CH_3CO), 24.0 [J (^{13}C–^{125}Te) = 150 Hz, $TeC^{2}H_2$], 61.6 ($C^{2}H_2OAc$), 121.2 [q, J (^{13}C–^{19}F) = 318.4 Hz, CF_3SO_3], 174.6 ($COCH_3$).

Storage of compounds

All pure compounds were stored under nitrogen at −70°C. Dilutions of these compounds that

were used for assay analysis were stored at either −45°C or −70°C under nitrogen. The pure compounds were stable for at least 6 months at −70°C, as were the dilutions.

Sample preparation and quantitation

The assay protocol used in the present study was based on the method of Terry *et al.* [10]. Briefly, the tissue sample to be assayed was placed in a 15-ml centrifuge tube and 2.5 nmol of deuterated (d_4-) telluronium choline and acetyltelluronium choline were then added as internal standards. A 2-ml volume of 1.0 *M* formic acid–acetonitrile solution (15:85, v/v) was added, and the sample was homogenized using a Polytron. The tube was centrifuged at 26 000 *g* for 20 min, and the supernatant was then transferred to 10-ml screw-capped centrifuge tube. A 4-ml volume of diethyl ether was then added to the tube, vortexed for 2 min, centrifuged for 2 min and the diethyl ether layer was then removed by aspiration. Traces of diethyl ether and ACN were evaporated under a vigorous stream of gaseous nitrogen for 5 min in a 25°C water bath. The telluronium compounds were then ion-pair extracted from the aqueous layer by adding 2 ml of 2 m*M* dipicrylamine (DPA) in methylene chloride, followed by 0.5 ml of 3-{[tris(hydroxymethyl)methyl]amino}-1-propanesulfonic acid (TAPS) buffer (pH 9.2). The tube was immediately vortexed for 2 min, centrifuged for 2 min, and the methylene chloride layer containing the ion-paired telluronium compounds was then transferred to a clean centrifuge tube. After evaporation of the methylene chloride layer to dryness under nitrogen at 25°C, the residue was dissolved in 250 μl of sodium acetate buffer (0.05 *M*, pH 4.0), and washed with 500 μl of 2-butanone (two times) to remove traces of DPA. The 2-butanone layer was aspirated, the aqueous layer was then evaporated to dryness under nitrogen in a 25°C water bath, and the tube stored on ice until used. Immediately prior to pyrolysis, 100 μl of acetonitrile–water (90:10) containing 250 μg of tetramethylammonium bromide [18] (TMABr) was added to the tube and then vortexed briefly. A 2-μl volume of the resulting mixture was applied to a 25 mm × 2 mm I.D. quartz tube, air dried, and then inserted into the pyrolysis interface for GC–MS analysis.

To allow quantitation, standard solutions of the d_0- and d_4-telluronium compounds were dissolved in 0.05 *M* sodium acetate (pH 4.0). Samples which contained 2.5 nmol of d_4-telluronium choline and d_4-acetyltelluronium choline as internal standards, together with known amounts (20 pmol–20 nmol) of the d_0 compounds, were then processed as described above for tissue samples. The d_0/d_4 ratios of the corrected areas (see *Spectroscopic studies)* were then plotted *versus* the d_0 analogue content to obtain a standard curve. Quantitation of "unknown" samples was accomplished by referencing the d_0/d_4 ratio to the standard curve.

RESULTS AND DISCUSSION

The present study demonstrates the successful synthesis of the novel choline analogues telluronium choline (TeCh) and acetyltelluronium choline (ATeCh). The overall synthetic yield for TeCh and ATeCh was 73 and 52%, respectively. Proof of the synthesis and purity of these analogues was provided by multinuclear NMR data, positive ion fast atom bombardment (Fig. 1), and elemental analysis (see Experimental).

Quantitation of the telluronium compounds was accomplished by modifying an existing pyrolysis–GC–MS procedure [10]. However, unlike the existing assay, telluronium choline and acetyltelluronium choline were not subjected to vacuum desiccation at any step during the assay since the related choline analogue, selenonium choline, spontaneously demethylated under these conditions. Unlike the selenonium analogues, the telluronium analogues also appeared to be thermally labile. The use of temperatures higher than 25°C during the evaporation steps resulted in significant losses of the analogues. Preliminary data (not shown) also indicate that the telluronium compounds are not stable for prolonged times at room temperature. The majority of the decomposition appeared to occur during the first 8 h and approximated 8–10% after 24 h. Because of this potential degradation, all samples were kept on ice except during the analytical processing steps.

Another major difference from the selenonium assay [10] was that propionylation of the alcohol function of TeCh was not required prior to GC–MS analysis since good peak separation was observed between ATeCh and TeCh. Further, the peak shape of the alcohol (TeCh) was symmetrical with peak widths ranging from 0.04 to 0.05 min at half-height. It was also observed that the procedure used for the propionylation of selenonium choline degraded both telluronium compounds to a significant extent, and reduced absolute recoveries.

Because the telluronium analogues were cationic, the compounds required demethylation prior to GC–MS analysis. The work of Terry *et al.* [10] previously demonstrated that it was possible to chemically demethylate the related selenonium analogues to a slight extent (*ca.* 10%) using sodium thiophenoxide. However, chemical demethylation of the telluronium compounds with this agent was ineffective. Based on the chemical similarity of the telluronium analogues to the selenonium compounds, other modes of chemical demethylation were not attempted, and thermal demethylation (pyrolysis) was selected. It was necessary to add approximately 250 μg of tetramethylammonium bromide (TMABr) to each sample prior to the final reconstitution step to aid in the pyrolysis of nanomolar quantities of the telluronium analogues. A similar approach has been used for the pyrolysis of picomolar quantities of choline and acetylcholine where only 5 μg of TMABr was sufficient [18]. The present data (not shown) indicate that the optimum pyrolysis temperature for the telluronium analogues was approximately 10–20°C lower than for the related selenonium analogues. The lower pyrolysis temperature requirement is compatible with the expected reduced stability of the tellurium–carbon bond compared to the analogous selenium–carbon bond.

Shown in Fig. 2 is a representative mass spectral "scan" for d_0- and d_4-TeCh after thermal demethylation, showing the characteristic clusters of fragments corresponding to the multiple isotopes of tellurium. As can be seen, demethylated TeCh did not fragment well, causing the molecular ion m/z 190 and m/z 194 to be

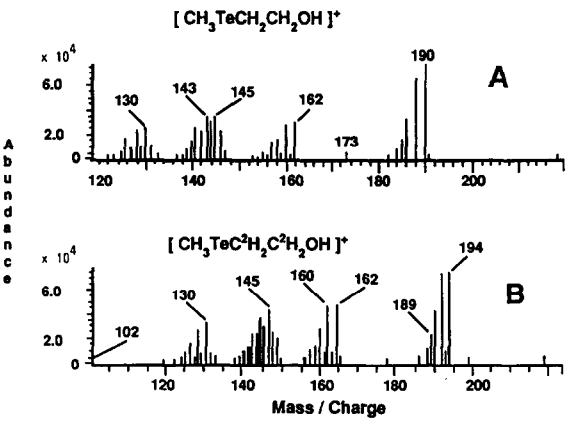

Fig. 2. Mass spectra of (A) telluronium choline and (B) [$^2H_4$]telluronium choline. The telluronium compounds were subjected to the described assay protocol and analyzed by pyrolysis–GC–MS. Data were obtained using the HP5890/5970 GC–MS system operated in the "scan" mode.

also the base peak for the d_0 and d_4 variants, respectively. Other major fragments included m/z 143 and 145 ($[CH_3Te]^+$) and m/z 130 ($[Te]^+$) which were produced by both analogues. Major fragments at m/z 160 and 162 appeared in both of the spectra for the TeCh analogues. Since the d_4 analogue also yielded the same fragments, this result suggested that during the fragmentation, an ethylene group was lost with the formation of a fragment corresponding to $[CH_3TeOH]^+$.

Shown in Fig. 3 is the "scan" obtained for the ATeCh analogues after thermal demethylation. The molecular ions (m/z 232 and 236) corresponding to the demethylated variants, were also the base peaks for the d_0 and d_4 analogues, respectively. The fragments at m/z 172 and 175 corresponded to $[CH_3TeCH=CH_2]^+$ and $[CH_3TeC^2H=C^2H_2]^+$ from the d_0 and d_4 spectra, respectively. The observed fragmentation patterns were significantly different from the selenonium analogues where the base peak was the fragment ($[CH_3SeCH=CH_2]^+$) common to both selenonium analogues [10]. A similar fragment (m/z 58, $[CH_3NCH=CH_2]^+$) was also observed for choline and acetylcholine [16].

The necessity for isotopic corrections prior to data analysis becomes evident on inspection of a representative matrix table used in the quantitation procedure (Table I). The spillover is due in

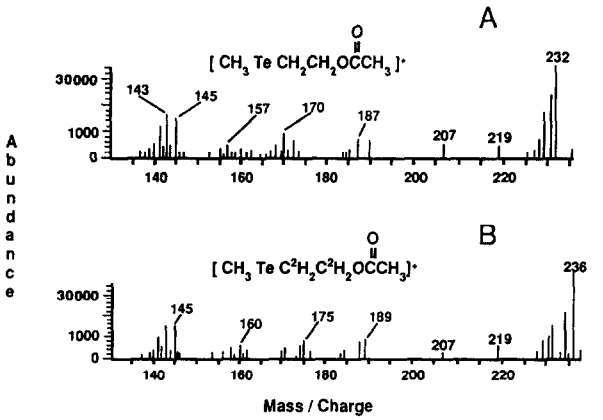

Fig. 3. Mass spectra of (A) acetyltelluronium choline and (B) [$^2H_4$]acetyltelluronium choline. The telluronium compounds were processed according to the described assay protocol and analyzed by pyrolysis–GC–MS. Data were obtained using the HP5890/5970 GC–MS system operated in the "scan" mode.

part to the large number of isotopes of tellurium and to the small difference in molecular mass between the d_0 and d_4 variants (4 u). For example, during fragmentation of demethylated d_4-ATeCh, a *m/z* 232 fragment is produced that contributes area to the base peak (*m/z* 232) of demethylated d_0-ATeCh (Fig. 3). From Table I it can be seen that the d_0 compounds contributed approximately 1–2% spillover into the areas of the d_4 analogues. However, approximately 53–55% of the d_4 areas were contributed to the area of the d_0 analogues. In comparison, the spillover of the d_4 analogues into the areas of the d_0 selenonium analogues was approximately 16–17% [10].

Fig. 4 is a representative chromatogram of 100 pmol of d_0-TeCh and d_0-ATeCh together with 2.5 nmol each of d_4-TeCh and d_4-ATeCh (internal standards). The four ions of interest were extracted from the total ion chromatogram using the "Ion Profile" software feature. The retention times for ATeCh and TeCh were approximately 14.1 and 14.7 min, respectively. For comparison, acetylselenonium choline eluted approximately 1 min earlier under the same conditions (data not shown). The abundance for the d_0 analogues was enhanced relative to the d_4 areas and, as discussed above, was due to the large spillover from the d_4 analogues.

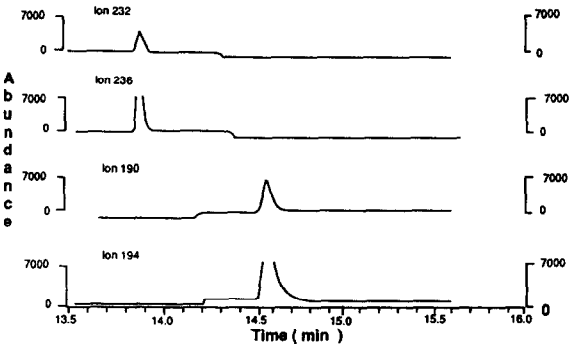

Fig. 4. Selected ion chromatogram of telluronium choline (TeCh) and acetyltelluronium choline (ATeCh). A 100-pmol amount of the d_0 analogues together with 2.5 nmol of the d_4 analogues were processed according to the described assay protocol and analyzed by pyrolysis–GC–MS. The ion chromatograms represent the ions of interest; *m/z* 232 (d_0-ATeCh), *m/z* 236 (d_4-ATeCh), *m/z* 190 (d_0-TeCh) and *m/z* 194 (d_4-TeCh).

Fig. 5 illustrates the linearity of the standard curve for 20 pmol through 20 nmol of d_0-TeCh and d_0-ATeCh. At the lowest extreme (20 pmol) and highest extreme (20 nmol), linearity appeared to fall off with increasing standard error. Since the matrix corrections were large, lack of linearity may be due to under- or overcorrection of the areas since only a single concentration (2.5 nmol) was used as the reference point for the calculations. Overcorrection of the areas also explains the negative ratio obtained for the 20

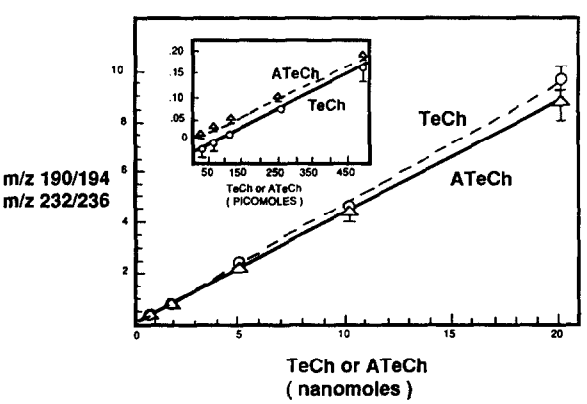

Fig. 5. Standard curves for telluronium choline (TeCh) and acetyltelluronium choline (ATeCh). Known quantities of telluronium analogues with 2.5 nmol of internal standard (d_4 telluronium variants) were processed using the described assay protocol and analyzed by pyrolysis–GC–MS. *m/z* 190/194 = TeCh, *m/z* 232/236 = ATeCh.

TABLE II

ASSAY REPRODUCIBILITY IN THE PRESENCE AND ABSENCE OF TISSUE

Known quantities of telluronium choline and acetyltelluronium choline in the presence and absence of 100 mg of mouse brain tissue were processed according to the protocol described and analyzed by pyrolysis–GC–MS.

Compound	pmol added	Observed (pmol)			
		Control	*n*	Brain tissue	*n*
Telluronium choline	100	118.3 ± 12	6	133.9 ± 17	6
	10 000	9230 ± 198	6	8970 ± 153	6
Acetyltelluronium choline	100	98.6 ± 27	6	79.9 ± 19	4
	10 000	9730 ± 169	6	9940 ± 186	6

pmol point for d_0-TeCh (Fig. 5, inset). For quantities to be analyzed at these extreme ranges of the standard curve, the use of matrix samples with quantities closer to the quantity to be measured should eliminate these problems.

Table II illustrates the recovery of the telluronium compounds in the presence of tissue. Considering the variability associated with pyrolysis methods [19], the data demonstrate a reasonable accuracy and standard error. For the TeCh data, the 100 pmol recoveries were higher than theoretical, and the 10 000 pmol quantities were lower than theoretical. The data for ATeCh were much closer to theoretical for both 100 and 10 000 pmol, but were generally lower than theoretical. All of the mean recovery values for both TeCh and ATeCh were within the standard error limits of the standard curve.

In conclusion, the compounds acetyltelluronium choline and telluronium choline have been synthesized in reasonable yield and high purity. These compounds can be quantitated in the presence of tissue by the use of pyrolysis GC–MS with linearity from 20 pmol to 20 nmol. The practical limit for the routine quantitation of these compounds using the described method is 50 pmol to 10 nmol due to the standard error experienced at concentrations outside this range.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Emily F. Willingham for her expertise in the preparation of this manuscript, and Dr. Robert L. Beamer, Chairman of the Department of Basic Pharmaceutical Sciences, College of Pharmacy, for his generous support provided for supplies. This research was also supported by the grants NSF No. BNS-8515929 (J.W.K.) and NIH GM42907 (J.D.O.). Support of high-field NMR spectrometer purchases at the University of South Carolina by the grants NSF (CHE 84-11172) and NIH (1-S10-RR02425) is also acknowledged. We would also like to thank the National Institutes of Health (RR 02231-10) Stable Isotopes Resource (SIR) at Los Alamos National Laboratory for partial financial support for this work (L.A.S.).

REFERENCES

1 A.D. Welch and M.H. Roepke, *J. Pharmacol. Exp. Ther.*, 55 (1935) 118.
2 E.M. Meyer, R.J. Barrnett and J.R. Cooper, *J. Neurochem.*, 39 (1982) 321.
3 L. Frankenberg, G. Heimburger, C. Nilsson and B. Sorbo, *Eur. J. Pharmacol.*, 23 (1973) 37.
4 B. Hedlund, H. Norin, A. Christakopoulos, P. Alberts and T. Bartfai, *J. Neurochem.*, 39 (1982) 871.
5 B. Collier, P. Boksa and S. Lovat, in F. Tucek (Editor), *The Cholinergic Synapse* (*Progress in Brain Research*, Vol. 49), Elsevier, Amsterdam, 1979, p. 107.
6 T.A. Patterson, A.V. Terry, Jr. and J.W. Kosh, *Br. J. Pharmacol.*, 97 (1989) 451.
7 A.V. Terry, Jr., L.A. Silks III, R.B. Dunlap, J.D. Odom and J.W. Kosh, *Gen. Pharmacol.*, 23 (1992) 689.
8 R.M. Dick, J.J. Freeman and J.W. Kosh, *Life Sci.*, 36 (1985) 1183.
9 P.T. Henderson, E.J. Ariens, B.W.J. Ellenbroek and A.M. Simonis, *J. Pharm. Pharmacol.*, 20 (1968) 26.

10 A.V. Terry, Jr., L.A. Silks III, R.B. Dunlap, J.D. Odom and J.W. Kosh, *J. Chromatogr.*, 585 (1991) 101.
11 J.N. Khatri, L.A. Silks III, R.B. Dunlap, J.D. Odom and J.W. Kosh, *Life Sci.*, 51 (1992) PL213.
12 R.P. Beliles, in L.J. Casarett and J. Doull (Editors), *Toxicology: The Basic Sciences of Poisons, Metals*, Macmillan, New York, 1975, p. 493.
13 J.O. Boles, *Ph.D. Dissertation*, University of South Carolina, Columbia, SC, 1992.
14 J.M. Iriarte, L.A. Silks and J.D. Odom, presented at the *Southeastern Regional American Chemical Society Meeting, Atlanta, GA, Nov. 1988*, Inorganic Section, abstract No. 415.
15 J.M. Iriarte, *Ph.D. Dissertation*, University of South Carolina, Columbia, SC, 1990.
16 D.J. Jenden and R.W. Silverman, *J. Chromatogr. Sci.*, 11 (1973) 601.
17 W.G. Kofron and L.M. Baclawski, *J. Org. Chem.*, 41 (1976) 1879.
18 R.L. Polak and P.C. Molinar, *J. Neurochem.*, 23 (1974) 1295.
19 J.Q. Walker, *Chromatographia*, 5 (1972) 547.