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Note

Determination of two nitrosourea antitumor agents by chemical ionization gas chromatography—mass spectrometry

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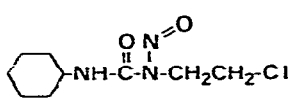
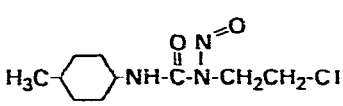
Two nitrosoureas, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (MeCCNU), are clinically useful for the treatment of neoplastic diseases. The quantitative determination of these drugs in biological samples requires high sensitivity combined with selectivity because of their rapid decomposition in aqueous media. Previous assays used for nitrosoureas include high-performance liquid chromatography [1], colorimetry [2—4], radiochemical analysis of labelled compounds [5, 6], differential pulse polarography [7], chemical ionization mass spectrometry with direct probe [8] and gas chromatography—mass spectrometry (GC—MS) [9]. The GC—MS method, using electron ionization (EI) of their trifluoroacetyl derivatives, combines the sensitivity of radiochemical analysis with the selectivity necessary to distinguish the parent drugs from their decomposition products. This assay has been used to study the pharmacologic disposition of MeCCNU [10]. Occasional samples, however, give erroneous results because of interfering components. Modification of this method for chemical ionization (CI) should increase the selectivity for these drugs.



CCNU



MeCCNU

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CI generally imparts less energy than EI to the sample molecule, resulting in less fragmentation and, in general, a greater proportion of ions relating to the intact molecule. Although a careful study indicated the relative sensitivities of EI and CI are essentially equal [11] the reduced fragmentation in the CI process significantly decreases contaminant interference in selected ion chromatograms of biological samples. This increased selectivity should in effect lower the limits of quantitation for these samples. This paper describes the chemical ionization of CCNU and MeCCNU by several reagent gases and its use for improving the assays for these agents.

MATERIALS AND METHODS

CCNU and MeCCNU were provided by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Standard solutions of these drugs were prepared from weighed samples by serial dilutions in methylene chloride. Trifluoroacetic anhydride and acetonitrile were obtained from Pierce (Rockford, IL, U.S.A.).

Plasma samples were extracted and prepared as previously described [9]. Variable volumes of urine (1—30 ml) were extracted twice with one-half volumes of diethyl ether—hexane after adding the internal standard. The extracts were combined, dried over anhydrous magnesium sulfate and the solvents evaporated under a nitrogen stream before derivatizing as described [9].

Instrumentation

Mass spectra and quantitative determinations were obtained from a Finnigan Model 3300F gas chromatograph—mass spectrometer with chemical ionization capability and interfaced with an Incos 2300 data system. The 1.2 m × 2 mm glass gas chromatographic column was packed with chemically bonded Carbowax 20M (Ultrabond 20M, RFR Corp., Hope, RI, U.S.A.). Chemical ionization mass spectra and selected ion chromatograms were obtained using methane, isobutane and ammonia reagent gases. Isobutane and ammonia were added as a make-up gas to the nitrogen carrier gas while methane also served as the chromatographic carrier gas. EI and CI data were obtained at 70 and 90 eV respectively.

RESULTS AND DISCUSSION

The mass spectra of trifluoroacetylated CCNU under EI, methane CI, isobutane CI, and ammonia CI conditions are compared in Fig. 1. The corresponding spectra of MeCCNU are very similar. As expected the mass spectra show less fragmentation in the order EI > methane CI > isobutane CI > ammonia CI. Surprisingly, the relative intensities of the protonated molecular ions in the methane and isobutane CI spectra are not significantly enhanced over that of the EI molecular ion, precluding their use in selective ion monitoring. However, the base ion in the ammonia CI spectrum is the ammonium adduct ion at *m/z* 414. Fig. 2 shows proposed decomposition pathways to account for the major ions derived from trifluoroacetylated CCNU under methane CI conditions.

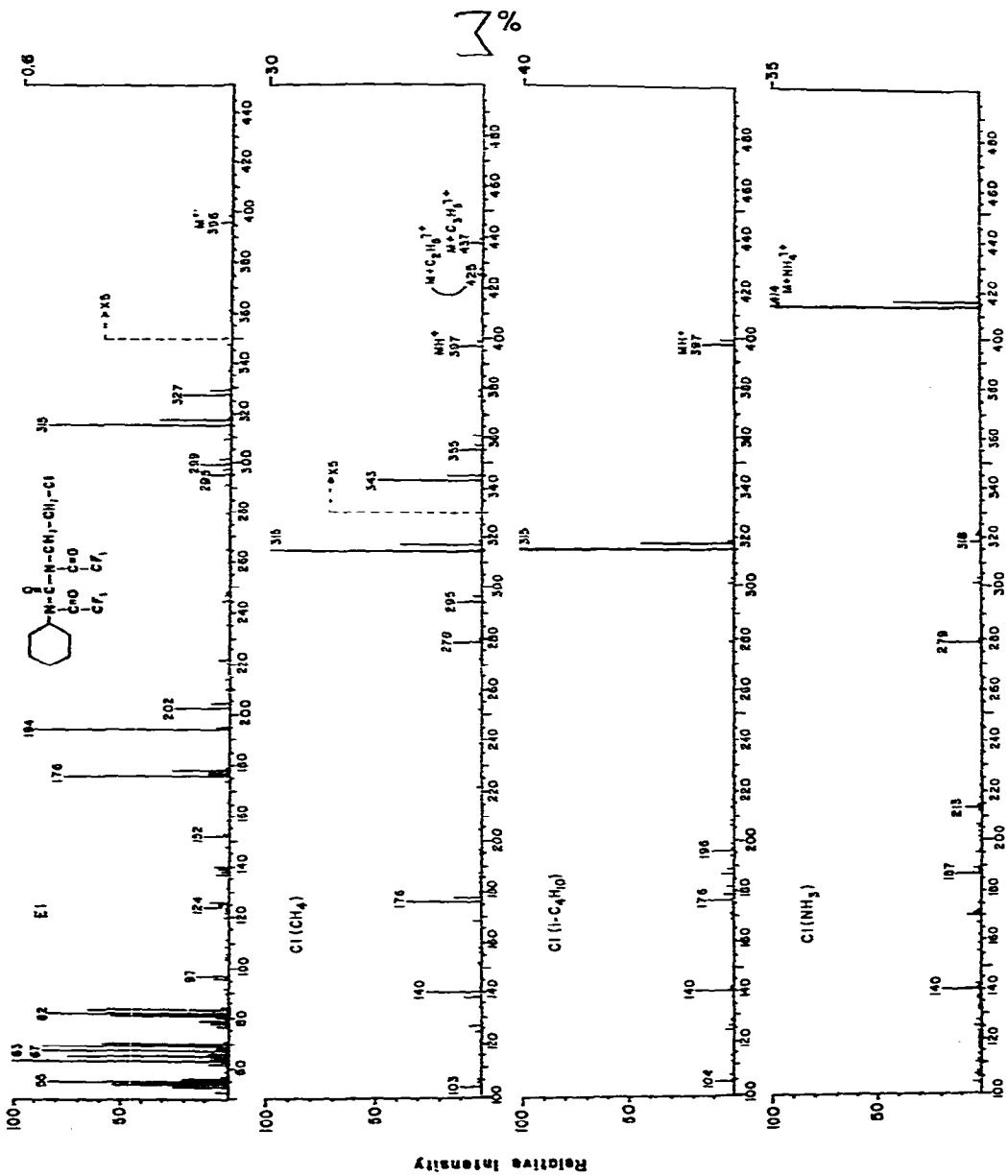

Fig. 1. Mass spectra of CCNU using EI, methane CI, isobutane CI and ammonia CI.

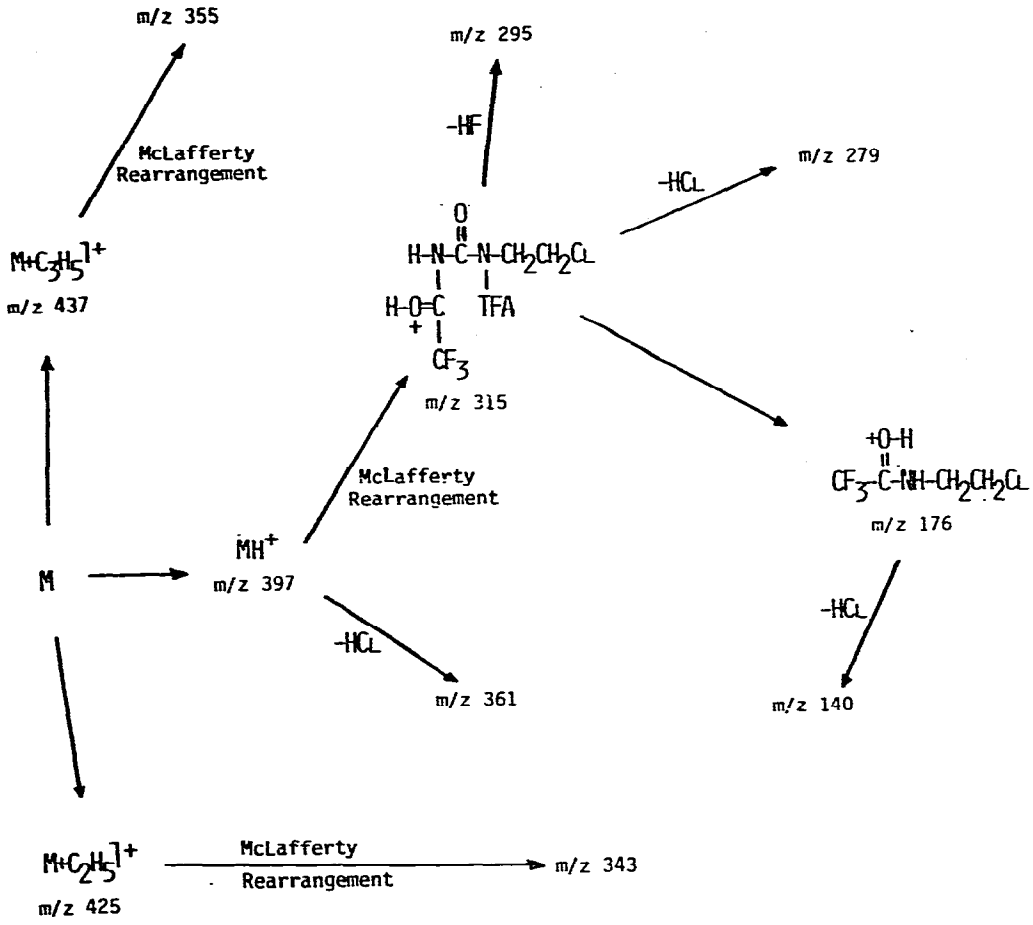

Fig. 2. Major decomposition pathways observed for CCNU after methane CI.

Selective ion monitoring at *m/z* 315 gives adequate sensitivity under EI conditions but interfering components from a few samples have necessitated changing to ions at *m/z* 317 or *m/z* 194. Despite the greatly reduced fragmentation with isobutane and ammonia reagent gases the sensitivity with these reagent gases was unsatisfactory. However, when monitoring *m/z* 315 with methane CI the responses for CCNU and MeCCNU appear slightly increased over those observed from EI. More important, the interference in the ion current chromatograms from extracted plasma is significantly reduced. Fig. 3 shows a selected ion chromatogram (*m/z* 315) of plasma containing 1.0 ng/ml of CCNU and 100 ng/ml of MeCCNU. The injected quantities were approximately 300 pg and 30 ng of CCNU and MeCCNU, respectively. A plasma blank exhibits no significant signal at the retention times of these two components. That the interference from plasma is minimal is also evidenced by a linear response over the drug concentration range of 1.0 ng/ml to 1.0 μg/ml.

Methane CI selective ion monitoring is being applied to pharmacology studies of CCNU and MeCCNU in experimental animals. The urinary excretion of unchanged MeCCNU from a dog after an intravenous administration

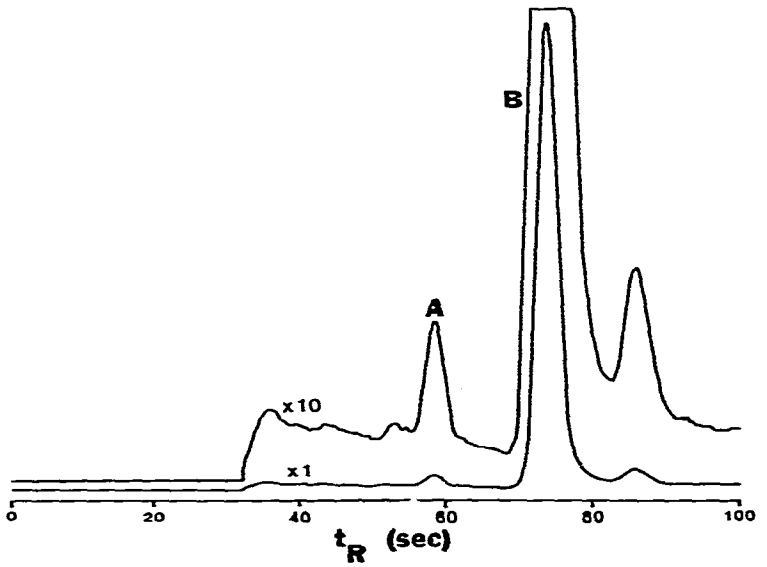

Fig. 3. Selected ion chromatogram (at *m/z* 315) for a plasma sample containing 1.0 ng/ml of CCNU (A) and 100 ng/ml of MeCCNU (B).

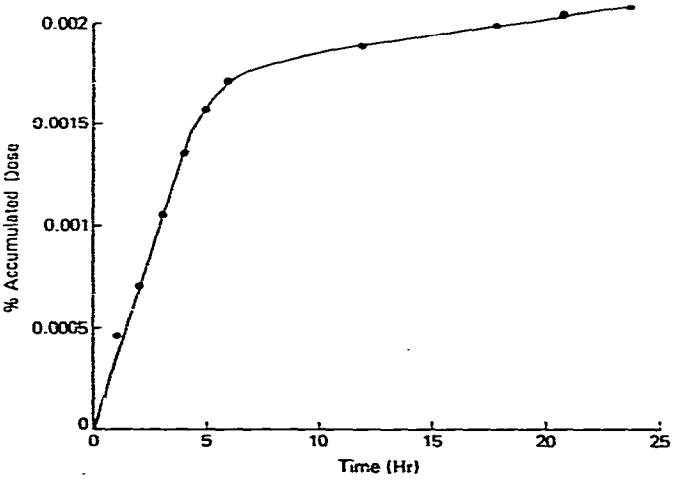

Fig. 4. Urinary excretion of MeCCNU from a dog after an intravenous administration of 15 mg/kg of this drug.

of 15 mg/kg of the drug is shown in Fig. 4. The excretion of the unchanged drug in minor, approximately 0.002% of the administered dose in 24 h. This is a dramatic contrast to the 50—60% of dose excreted by patients as measured by radioactivity [6]. This discrepancy is due to the nonspecific nature of the radiochemical assay and empnasizes the need for more definitive methodology.

CONCLUSION

Modification of the sensitive GC—MS selected ion monitoring assay for

CCNU and MeCCNU to use methane CI provides even greater sensitivity and selectivity; a ten-fold increase in sensitivity is realized by reducing the background signal due to endogenous components. Although this modification has not yet been applied to 1,3-bis(2-chloroethyl)-1-nitrosourea, it is reasonable to expect a similar improvement.

REFERENCES

1 I.S. Krull, J. Strauss, F. Hochberg and N.T. Zervas, J. Anal. Toxicol., 5 (1981) 42.
2 T.L. Loo and R.L. Dion, J. Pharm. Sci., 54 (1965) 809.
3 S.S. Mirvish, J.P. Sams and S.D. Arnold, Z. Anal. Chem., 298 (1979) 408.
4 P. Kari, W.R. McConnell, J.M. Finkel and D.L. Hill, Cancer Chemother. Pharmacol., 4 (1980) 243.
5 V.T. Oliverio, W.M. Vietzke, M.K. Williams and R.A. Adamson, Cancer Res., 30 (1970) 1330.
6 R.W. Sponzo, V.T. DeVita and V.T. Oliverio, Cancer, 31 (1973) 1154.
7 I. Bartosek, S. Daniel and S. Sykora, J. Pharm. Sci., 67 (1978) 1160.
8 R.J. Weinkam, J. Wen, D.E. Furst and V.A. Levin, Clin. Chem., 24 (1978) 45.
9 R.G. Smith, S.C. Blackstock, L.K. Cheung and T.L. Loo, Anal. Chem., 53 (1981) 1205.
10 R.G. Smith, S.C. Blackstock, L.K. Cheung, G.L. Raulston, J.P. Chang and T.L. Loo, Proc. Amer. Assoc. Cancer Res., 22 (1981) 270.
11 B. Munson, Anal. Chem., 49 (1977) 772A.