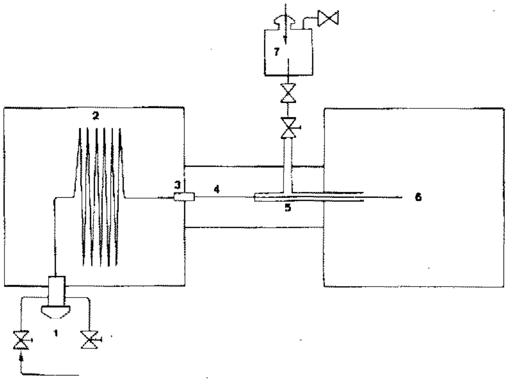
Tetrahedron Letters No. 7, pp 565 - 568, 1976. Pergamon Press. Printed in Great Britain.

ON-LINE HYDROGEN/DEUTERIUM EXCHANGE IN CAPILLARY GAS CHROMATOGRAPHY - CHEMICAL IONIZATION MASS SPECTROMETRY (GC-CIMS) AS A MEANS OF STRUCTURE ANALYSIS IN COMPLEX MIXTURES +)

W. Blum, E. Schlumpf, J. G. Liehr and W. J. Richter *
Zentrale Funktion Forschung, CIBA-GEIGY AG, Basel, Switzerland

(Received in UK 20 November 1975; accepted for publication 7 January 1976)

Deuterium exchange of acidic hydrogen atoms in organic molecules has been effected by Hunt et al. [1] in selected single compounds by means of chemical ionization (CI), using D_2O as a reagent gas. Excellent exchange efficiency was obtained by direct sample insertion even when as many as five acidic protons were present in the molecules. Since the determination of the number of exchangeable hydrogen atoms yields a characteristic, structurally informative parameter, extension of this work towards the analysis of constituents of mixtures by GC-MS techniques without previous isolation, appeared highly desirable. The following diagram shows the dual-gas glass capillary GC-CIMS system which we adopted for exploring this possibility. It proved capable of performing, with the same high quality, on-line H/D exchange on complex mixtures.



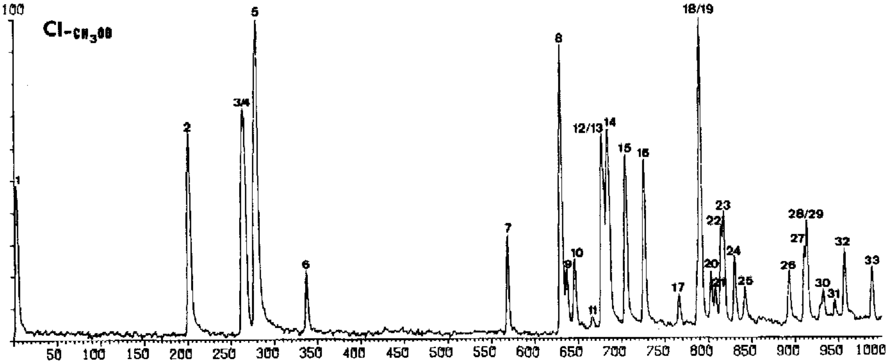
+) Presented in part at the Twenty-third Annual Conference on Mass Spectrometry and Allied Topics, Houston, Texas, May 25-30, 1975.

With this system, independent optimal carrier and reagent gases (entering at the injection port (1) of the gas chromatograph and a heated reservoir for liquids [2] (7) to be used as CI reactants, respectively) can be chosen freely as the latter is admixed behind the glass capillary column (2), i.e. after separation is effected. Thus, the retention characteristics of the components of the mixture remain unchanged when switching from one reagent gas to another. Direct coupling of the thin-film capillary (2) is afforded via a Polyimide ferrule [3] (3) and a platinum capillary [4] (4), which is part of a coaxial interface [5] (5) that serves the common admission of both gases, without prior mixing, to the CI source of a FINNIGAN 3300 quadrupole mass spectrometer (6). Polar reagent gases such as water or methanol and, for H/D-exchange, their deuterated analogs, can thus be admitted without interacting unfavorably with the inside wall coating of the interface capillary (4). These coatings are built up, during initial use, by stationary phase bleeding from the column, and are essential for proper operation of the system.

In order to assess the performance of this system with respect to GC resolution and quality of exchange, a mixture of o-, m- and p-phenylenediamine was subjected, in two separate experiments, to non-exhaustive N-methylation and ethylation with excess methyl or ethyl iodide in presence of KOH, respectively. The reactions were quenched after short times by addition of acid. Combining the reaction products was expected to furnish a suitable multi-component system of isomers and homologs containing no less than 33 constituents assuming that, in addition to unreacted starting materials, all possible methyl and ethyl derivatives were present. The objective was, to generate a multitude of closely related compounds which would lend themselves to convenient classification as to degree and kind of alkylation, simply by determining their number of exchangeable hydrogen atoms and molecular weights.

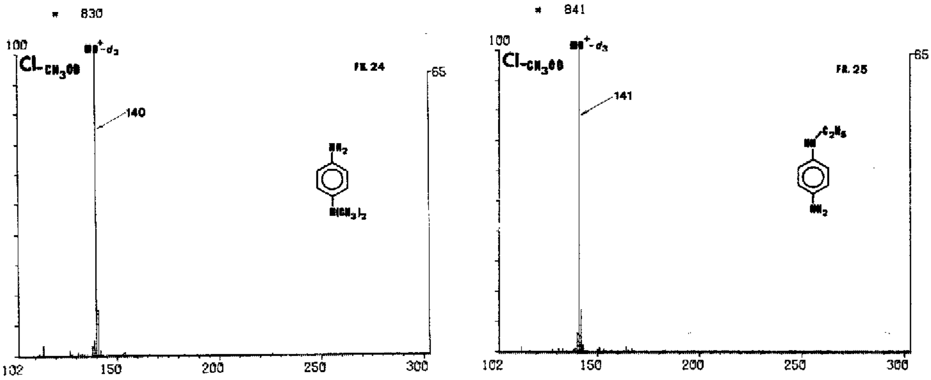
The following figure shows a computer-reconstructed total-ion-current gas chromatogram (TIC-GC) obtained from this model mixture in a typical CI(CH_3OD) experiment. Comparison of this trace with an FID gas chromatogram recorded in a parallel GC run, i.e. without MS coupling, revealed that practically no loss of the high separation efficiency of the glass capillary column had occurred. For determining the number of exchangeable hydrogen atoms, spectra were continuously recorded and processed by a FINNIGAN 6000 data system during two successive analogous experiments, one employing CH_3OH, and one CH_3OD as reactant. Direct comparison of the CI(CH_3OH) spectra with the ones obtained by CI(CH_3OD) for the same GC fraction furnished, immediately, the nominal mass differences Δm between MH^+ and MD^+ quasimolecular ions, since they represented almost solitary signals, i.e. the only ions due to sample, formed under these conditions. In the present case of phenylenediamines, the number of exchangeable hydrogen atoms $n_{\underline{H}(acidic)} = \Delta m - 1$ yields the number of introduced alkyl substituents promptly as $n_{alkyl} = 4 - n_{\underline{H}(acidic)}$. Since total ion current is heavily concentrated in these quasimolecular ions, isomers such as monoethyl and dimethyl compounds can be reliably distinguished by these numbers, even when they are incompletely separated, or present in low abundances. Although these numbers allowed a straightforward classification of all components into methylated or ethylated isomers or homologs, an identification of the substitution type (o-, m-, and p-)

necessitated, however, supplementary alkylation experiments on the pure phenylenediamine isomers. N,N- and N,N'-disubstitution was distinguished through suitable reference compounds. The detailed analysis of the CI spectra disclosed that all 33 possible constituents were present in this mixture, although not all of them were completely resolved.



Total ion current gas chromatogram (TIC-GC) of methylated and ethylated phenylenediamines. Glass capillary column, 50 m x 0.36 mm, coated with Ucon HB 5100 and especially modified for separation of amines (H. & G. Jaeggi, Labor f. Gaschromatographie, CH-9043 Trogen, Switzerland). Linear temperature program 50 to 200°C, 2.4°C/min. Components: $\underline{o}$-$C_6H_4(NMe_2)_2$ (1); $\underline{o}$-$C_6H_4(NMe_2)N\underline{H}Me$ (2); $\underline{o}$-$C_6H_4(N\underline{H}Me)_2$ (3); $\underline{o}$-$C_6H_4(NEt_2)N\underline{H}Et$ (4); $\underline{o}$-$C_6H_4(NEt_2)_2$ (5); $\underline{o}$-$C_6H_4(N\underline{H}Et)_2$ (6); $\underline{p}$-$C_6H_4(NMe_2)_2$ (7); $\underline{o}$-$C_6H_4(NEt_2)N\underline{H}_2$ (8); $\underline{o}$-$C_6H_4(NMe_2)N\underline{H}_2$ (9); $\underline{m}$-$C_6H_4(NMe_2)_2$ (10); $\underline{o}$-$C_6H_4(N\underline{H}Me)N\underline{H}_2$ (11); $\underline{o}$-$C_6H_4(N\underline{H}Et)N\underline{H}_2$ (12); $\underline{o}$-$C_6H_4(N\underline{H}_2)_2$ (13); $\underline{p}$-$C_6H_4(N\underline{H}Me)_2$ (14); $\underline{p}$-$C_6H_4(NMe_2)N\underline{H}Me$ (15); $\underline{p}$-$C_6H_4(NEt_2)_2$ (16); $\underline{p}$-$C_6H_4(N\underline{H}Et)_2$ (17); $\underline{p}$-$C_6H_4(N\underline{H}_2)_2$ (18); $\underline{m}$-$C_6H_4(NEt_2)_2$ (19); $\underline{p}$-$C_6H_4(NEt_2)N\underline{H}Et$ (20); $\underline{p}$-$C_6H_4(N\underline{H}Me)N\underline{H}_2$ (21); $\underline{m}$-$C_6H_4(NMe_2)N\underline{H}Me$ (22); $\underline{m}$-$C_6H_4(N\underline{H}Me)_2$ (23); $\underline{p}$-$C_6H_4(NMe_2)N\underline{H}_2$ (24); $\underline{p}$-$C_6H_4(N\underline{H}Et)N\underline{H}_2$ (25); $\underline{p}$-$C_6H_4(NEt_2)N\underline{H}_2$ (26); $\underline{m}$-$C_6H_4(N\underline{H}_2)_2$ (27); $\underline{m}$-$C_6H_4(NEt_2)N\underline{H}Et$ (28); $\underline{m}$-$C_6H_4(N\underline{H}Et)_2$ (29); $\underline{m}$-$C_6H_4(N\underline{H}Me)N\underline{H}_2$ (30); $\underline{m}$-$C_6H_4(NMe_2)N\underline{H}_2$ (31); $\underline{m}$-$C_6H_4(N\underline{H}Et)N\underline{H}_2$ (32); $\underline{m}$-$C_6H_4(NEt_2)N\underline{H}_2$ (33).

The quality of exchange attained, can be judged from the following figure which shows the CI (CH_3OD) spectra of two $\underline{p}$-isomers, N,N-dimethyl-$\underline{p}$-phenylenediamine and N-ethyl-$\underline{p}$-phenylenediamine (components 24 and 25). The compounds are classified as isomers of molecular weight 136 by exhibiting m/e 137 quasimolecular ions MH^+, in the CI(CH_3OH) experiment. Corresponding MD^+ ions at m/e 140 and 141, respectively, characterize them as d_2- and d_3-compounds, and hence as dimethyl and monoethyl derivatives. Calculation reveals that total H/D exchange is better than 95%. As opposed to the use of less mildly protonating reagent gases such as CH_4 etc., molecular ions $M^{+\cdot}$, as possible products of charge exchange (CE), are largely absent when $CH_3OH(D)$ is used as reactant. Concomitant CE, to which donor-substituted aromatic systems like the ones studied are rather susceptible,[6] would be liable to interfere with evaluating



CI(CH_3OD) spectra of N,N-dimethyl-p-phenylenediamine and N-ethyl-p-phenylenediamine (GC fractions 24 and 25, respectively). Source temperature 120°C; reagent gas: CH_3OD (600 micron).

the results when low-concentration and/or overlapping components are analysed. Advantages of use of CH_3OH/CH_3OD instead of H_2O/D_2O in CI refer to lifetime expectancy of hot filaments in CI ion sources, and possibly to the prevention of H/D exchange of aromatic protons in the 2-, 4- and 6-positions of the meta-compounds. This additional exchange can range from negligible to essentially complete, depending on experimental conditions to be further investigated.

References:

1. D. F. Hunt, C. N. McEwen and R. E. Upham, Anal. Chem. 44, 1292 (1972).
2. W. Blum and W. J. Richter, to be published.
3. W. Blum and W. J. Richter, Helv. Chim. Acta 57, 1744 (1974).
4. N. Neuner-Jehle, F. Etzweiler and G. Zarske, Chromatographia 6, 211 (1973).
5. W. Blum and W. J. Richter, Finnigan Spectra 5/1 (1975).
6. B. S. Finkle, R. L. Foltz and D. M. Taylor, J. Chromatogr. Sci. 12, 304 (1974).