Analyses of Pyrimidine and Purine Bases by a Combination of Paper Chromatography and Time-of-Flight Mass Spectrometry

MARTIN H. STUDIER

Chemistry Division, Argonne National Laboratory
Argonne, Illinois 60439

and

RYOICHI HAYATSU and KIYONO FUSE

Enrico Fermi Institute, University of Chicago
Chicago, Illinois 60637

NsG-366

Running Title: Paper Chromatography - Mass Spectrometry

Proofs should be mailed to Martin H. Studier
Chemistry Division
Argonne National Laboratory
Argonne, Illinois 60439

Analyses of Pyrimidine and Purine Bases by a Combination of Paper Chromatography and Time-of-Flight Mass Spectrometry*

MARTIN H. STUDIER
Chemistry Division, Argonne National Laboratory
Argonne, Illinois 60439

and

RYOICHI HAYATSU and KIYONO FUSE
Enrico Fermi Institute, University of Chicago
Chicago, Illinois 60637

INTRODUCTION

In recent years a great deal of effort has been devoted to the isolation, identification and synthesis of various purine and pyrimidine derivatives. The occurrence of compounds of this type in meteorites and terrestrial deposits has been investigated because of biological significance and possible role in the origin of life (1). Because the quantity of material available is often very limited, specialized techniques for separation and detection must be used. Limited volatility of these bases makes

---

* Based on work performed in part under the auspices of the U.S. Atomic Energy Commission, and NASA (grant NSG-366).

gas chromatography an impractical method. A technique which has been highly successful is that of paper chromatography. A number of solvent systems have been described (2) for the separation of purine and pyrimidine bases by paper chromatography. However, the separation of complex mixtures of closely related compounds is often incomplete, and positive identification of components may be difficult (3). Comparisons with known compounds must be made, and unknown compounds remain unidentified. Combination with another technique would be useful. The combination of mass spectrometry with paper and thin layer chromatography for other types of compounds has been suggested but few experimental details are given (4).

We shall describe a promising technique for studying purine and pyrimidine bases by a combination of mass spectrometry and paper chromatography. Mass spectra of numerous bases have been published recently (5). In general these are characterized by simple spectra with prominent parent ions. Thus, mass spectrometric identification is relatively simple.

METHODS

Mass spectra were obtained with a modified (6) time-of-flight machine (Bendix Corporation). In all experiments the same chromatographic paper (Whatman 3MM) was used. All solvent systems tried gave satisfactory results except those with strong mineral acids.

1. n-butyl alcohol - acetic acid - water (2:1:1) and (4:1:2)
2. t-butyl alcohol - methyl ethyl ketone - water - formic acid (44:44:10:0.3)
3. n-propyl alcohol - 1M NH_4OH (67:100)

Solvent systems containing 2M HCl hydrolyzed so much paper that satisfactory mass spectra were not obtained until samples were purified further with another solvent system.

To test procedures authentic samples of about one microgram were spotted on chromatographic paper and developed with a solvent. After the paper was dried the bases were located by their characteristic strong absorption of short wave ultra-violet light. Sections of interest were cut out, placed on a microscope slide, and extracted with water or 0.1M HCl. (The mass spectra of the hydrochlorides gave the same mass spectra as the free bases plus that of HCl.) The extracts were evaporated on to thin platinum ribbons which were then placed into the source region of the mass spectrometer in a position close to the ionizing electron beam (6). Mass spectra were obtained as the filaments were heated electrically to distill the samples into the region of the ionizing electrons. The small heat capacity of the platinum ribbon makes the temperature instantly responsive to the applied voltage. Memory effects are at a minimum since the filament containing the sample is much warmer than the immediate surroundings. Usually twenty or thirty mass spectra were obtained with an oscillographic recorder while the entire spectrum was displayed on the screen of an oscilloscope.

In Figure 1 is shown a photograph of an oscilloscope display of the principle peaks in the mass spectrum of xanthine (not published previously) extracted with 0.1M HCl from a paper developed with n-butanol - acetic acid - H_2O (2:1:1). A picture of the background in the spectrometer taken within a second after the filament current was turned off is shown also. It was indistinguishable from the background before the sample was heated. The parent ion at mass 152 is slightly higher than the one at mass 109. At higher filament temperatures mass 54 became prominent also. The spectrum shown was taken with 70-volt electrons and changed little with energy.

RESULTS AND DISCUSSION

A practical test of the method is illustrated in Figure 2. A solution containing one microgram of each of eight bases (adenine, guanine, cytosine, hypoxanthine, 5-methylcytosine, thymine, uracil and xanthine) was spotted on paper and developed in two dimensions with two solvent systems under conditions deliberately chosen to give incomplete separation. Spots produced by absorption of ultraviolet light were outlined and numbered. Areas of only slight absorption were outlined with dashed lines. The compounds identified mass spectrometrically in each area are given.

A solution of a mixture of the eight bases of Figure 2 (one microgram each) was evaporated directly on a filament and analyzed mass spectrometrically. A computerized display of a fast scan mass spectrum is shown in Figure 3. The parent ion of each base is readily discernable. Guanine and xanthine are lower than others because they had partially precipitated from solution. Because of the uniqueness of the mass spectra and the small samples required, preliminary mass analyses prior to extensive separations may be useful.

In general, excellent mass spectra were obtained for bases extracted from paper developed with a variety of solvents (solvents with a 2M HCl excepted). Spectra were surprisingly free of interferences. Peaks at mass 64 (SO_2^+) and 48 (SO^+) were always observed from sulphite contaminants from the paper. Occasionally traces of solvents remained which were distilled away before the spectra of the bases appeared. The responsiveness of the system for heating samples permitted fractionation from impurities while the spectrum was observed on the oscilloscope.

The combination system of paper chromatography - mass spectrometry is being used successfully to study synthetic mixtures of bases as well as some isolated from natural products.

ACKNOWLEDGMENTS

The authors are grateful to Mrs. Alberta Martin and Mr. Leon P. Moore for technical assistance. We thank Dr. Anders for his interest and encouragement.

5/14/68

skj

REFERENCES

1. a) MAMIKUNAIN, G. and BRIGGS, M. H. (Editors) "Current Aspects of Exobiology," Pergamon Press, New York, 1965.

   b) SHENOUR, E. A. and OTTESON, E. A., "Extraterrestrial Life: An Anthology and Bibliography," National Academy of Sciences National Research Council, Washington, D.C., 1966.

   c) BREGER, I. A. (Editor) "Organic Geochemistry," MacMillan Company, New York, 1963.

2. FINK, K. and ADAMS, W. S., J. Chromatog. 22, 118 (1966).

3. HAYATSU, R., STUDIER, M. H., ODA, A., FUSE, K., and ANDERS, E., Geochim. Cosmochim. Acta 32, 175 (1967).

4. a) HEYNS, K. and GRUETZMOCHER, H. F., Angew. Chem. Internat. Edit. 1, 400 (1962)

   b) FETIZON, M., in "Thin Layer Chromatography," (Marini-Betolo, G. B., Ed.) p. 69, Elsevier, Amsterdam, 1964

   c) BIEMANN, K., "Mass Spectrometry," p. 192, McGraw-Hill, New York, 1962.

5. a) RICE, J. M., DUDEK, G. O., and BARBER, M., J. Am. Chem. Soc. 87, 4569 (1965)

   b) RICE, J. M. and DUDEK, G. O., J. Am. Chem. Soc. 89, 2719 (1967).

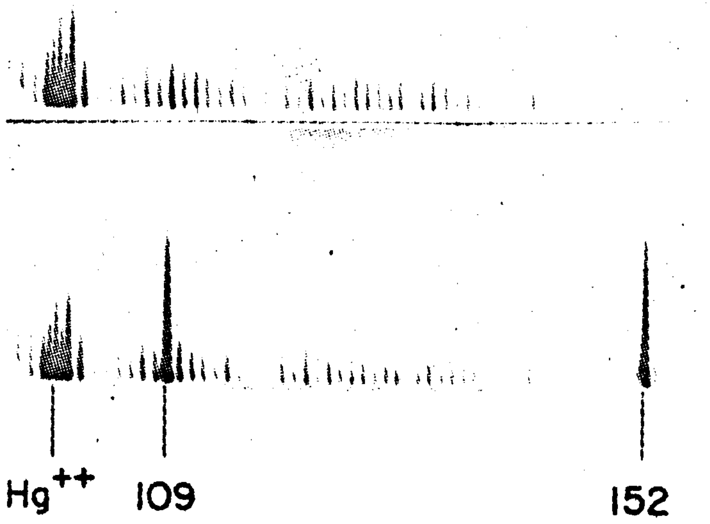
6. STUDIER, M. H., Rev. Sci. Instr. 34, 1367 (1963).

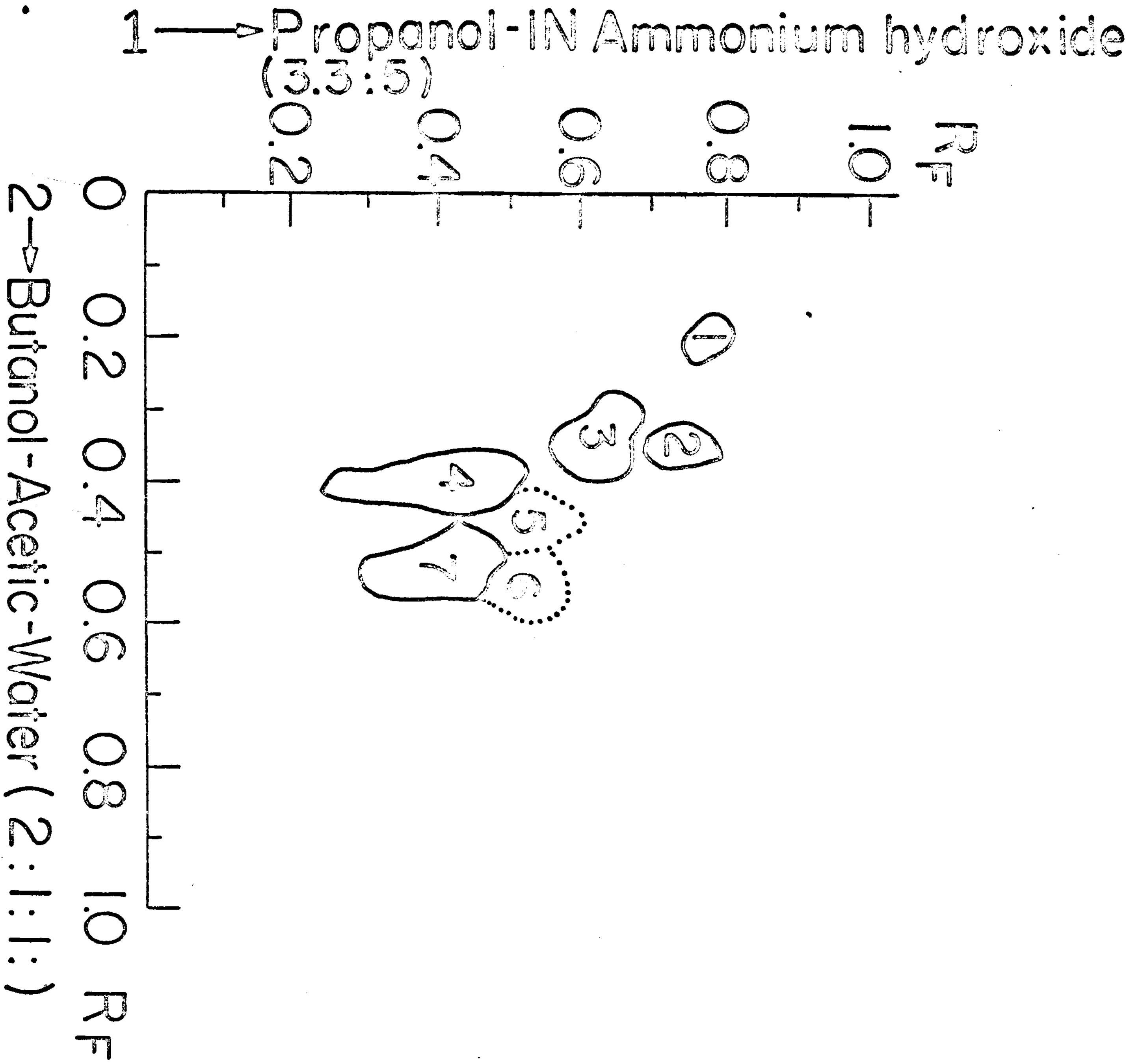
FIGURE CAPTIONS

FIG. 1. Bottom.--Mass spectrum of xanthine from sample extracted from chromatographic paper. Top.--Background spectrum one second after turning off sample filament.

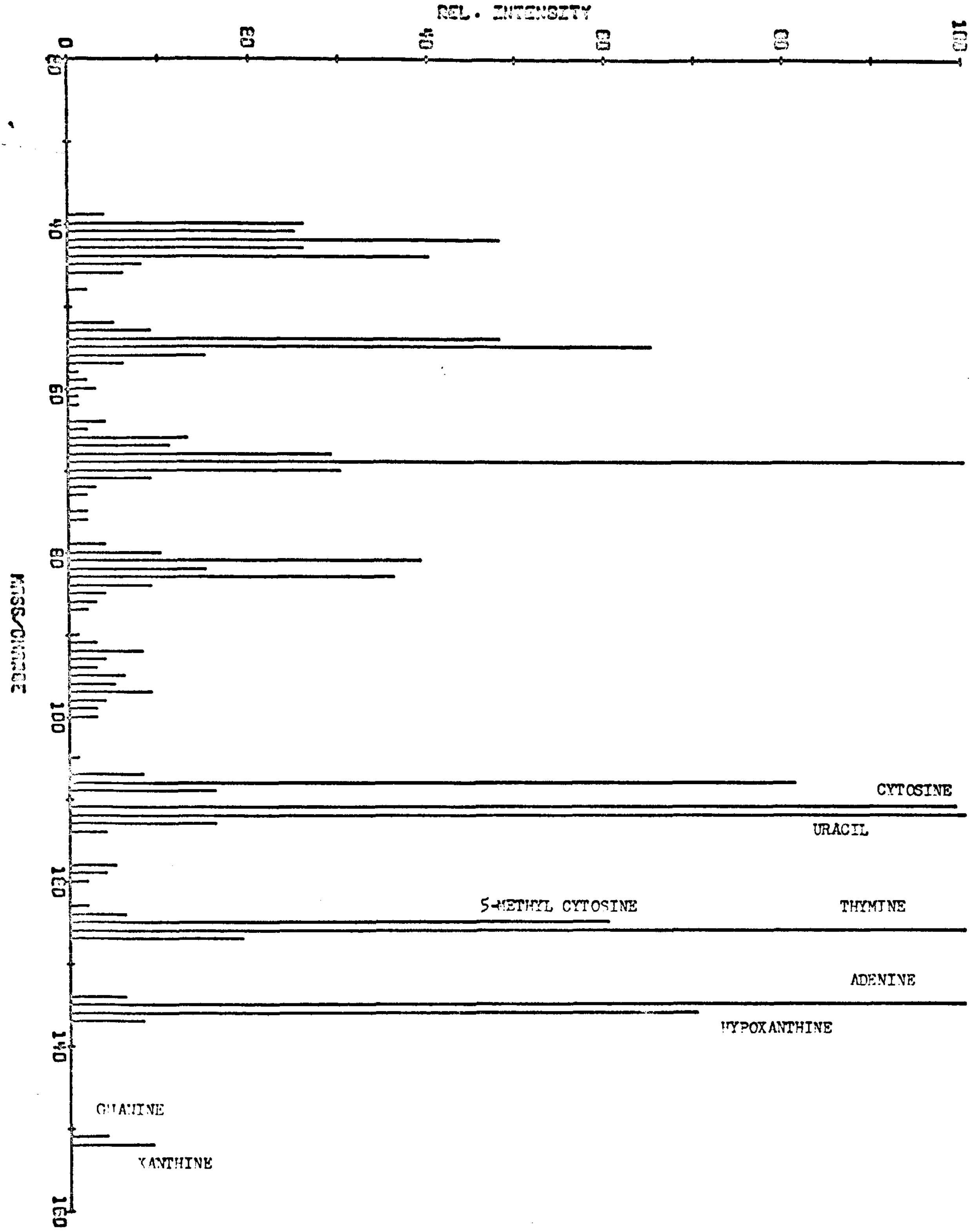
FIG. 2. Chromatogram of a mixture of eight bases. Identification of spots by mass analysis as follows: 1.—thymine, 2.—adenine, 3.—cytosine, 5 methylcytosine, uracil, hypoxanthine, 4.—xanthine, 5.—no significant mass spectra, 6.—trace guanine, 7.—guanine.

FIG. 3. Mass spectrum of a mixture of eight bases.


Hg^{++}
109
152


1→Propanol-IN Ammonium hydroxide (3.3:5)
2→Butanol-Acetic-Water (2:1:1:)
R_F
0
0.2
0.4
0.6
0.8
1.0
0
0.2
0.4
0.6
0.8
1.0
R_F
1
2
3
4
5
6
7

8-BASES


REL. INTENSITY
0
20
40
60
80
100
MASS/CHARGE
20
40
60
80
100
120
140
160
CYTOSINE
URACIL
5-METHYL CYTOSINE
THYMINE
ADENINE
HYPOXANTHINE
GUANINE
XANTHINE