On alkaline hydrolysis followed by diazomethane methylation, IV-B yielded smoothly 1-methyl-4-methoxyphenazine-6-methanol (VI), $C_{15}H_{14}O_2N_2$, mp 211°. NMR: 7.23 (3H, s), 5.94 (3H, s) (C_6H_5–$OC\underline{H}_3$), 4.69 (2H, s) (C_6H_5–$C\underline{H}_2$–OH), which lacks the carbonyl absorption band in its infrared (IR) spectrum. The hydroxymethyl derivative (VI) was then oxidized with potassium permanganate in acetone at reflux to afford an aldehydic compound (VII), $C_{15}H_{12}O_2N_2$, mp 211°, IR cm^{-1}: 1690. NMR: 7.22 (3H, s), 5.88 (3H, s), −2.03 (1H, s; assigned to the aldehydic hydrogen and was unaffected by the addition of D_2O), Mass Spectrum: 252 (M^+), in good yield. Further treatment of VII with potassium permanganate surprisingly was found to terminate mostly with the recovery of the starting aldehyde and with the trace amount of an acidic product. However, the conversion of the aldehyde function to an acid was successfully effected by silver oxide. Thus, treatment of VII in aqueous silver nitrate solution with alkali at room temperature for 1 hour furnished 1-methyl-4-methoxyphenazine-6-carboxylic acid (VIII), $C_{15}H_{12}O_3N_2$, mp 256°. IR cm^{-1}: 1720. NMR: 7.20 (3H, s), 5.89 (3H, s), in high yield. The methyl ester (IX), NMR: 7.21 (3H, s), 5.84 (6H, s), of VIII was subsequently subjected to N-bromosuccinimide bromination under irradiation followed by acetolysis as above giving a methyl ester acetate, mp 153°. IR (KBr) cm^{-1}: 1737, 1698, 1283, 1248. NMR: 7.88 (3H, s) (-OCOC$\underline{H}_3$), 5.91 (3H, s) (-COOC$\underline{H}_3$), 5.86 (3H, s) (C_6H_5-OC$\underline{H}_3$), 4.18 (2H, s) (C_6H_5-C$\underline{H}_2$-O-). The final product thus obtained was identified with methyl 1-acetoxymethyl-4-methoxyphenazine-6-carboxylate (X),[2a] mp 152°, prepared from the authentic sample of methyl griseoluteate (Ib) cordially supplied by Prof. S. Nakamura, in all respects (mixed mp, IR, TLC, NMR and Mass spectra). It should be noted here that in general on N-bromosuccinimide bromination[6] of the anisol derivatives, benzoyl peroxide tends to catalyze the nuclear bromination while under irradiation as in the present case the benzylic bromination takes place, which will be detailed in our full paper.

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Feasibility of Gas Chromatography for Ultra-micro Analysis of Aluminum in Biological Materials

Recently, gas chromatography has been extended to the study of inorganic fields such as metal chelates.[1] However, very little has been known for the application of gas chromatography to the analysis of ultra-micro amounts of metals which are present in biological materials. Meanwhile, the presence and the location of aluminum in human bodies have attracted much attention,[2] although biological significance of aluminum has not been clarified in detail. These facts described above stimulated us to investigate gas chromatographic analysis of aluminum in biological materials.

1) See for instance: R.S. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, Oxford, 1965.
2) I.H. Tipton, "Metal-Binding in Medicine," ed. by M.J. Seven, L. Audrey Johnson, J.B. Lippincott Company, Philadelphia, 1960, pp. 27—42.

In this communication, we wish to report some observation on the feasibility of gas chromatography for ultra-micro analysis of aluminum in a rat liver for an example.

22 g of a rat liver was minced and digested in hot H_2SO_4–HNO_3 medium by the usual wet method to destruct completely organic matters, and the digest was neutralized with NaOH and adjusted to pH 4—5, and made up to 50 ml by diluting with distilled water.

1 ml aliquot of the digest solution was transferred into a test tube with a ground glass stopper, and mixed with 2 ml of buffer solution of pH 4.5, and 1 ml of distilled water. To extract aluminum from the solution, 2 ml of 0.5% trifluoroacetylacetone–benzene was added and the mixture was vigorously shaken for 3 min by a shaking machine. After shaking, the water layer was discarded and the benzene layer was washed two times with each 4 ml of buffer solution of pH 9.3, and finally the benzene layer was submitted as a sample to gas chromatographic investigation.

As for the standard substance of gas chromatography, aluminum trifluoroacetylacetonate was synthesized by reacting anhydrous $AlCl_3$ with trifluoroacetylacetone (bp 107°) in dry CCl_4. The reagents used here were all of analytical grade.

Gas chromatography was performed by JGC 810 Gas Chromatograph (Japan Electron Optics Laboratory Co. Ltd.) equipped with a ^{63}Ni electron capture detector. The column was 3 mm (*i.d.*)×1.1 m stainless steel tubing packed with 3% OV-17 on Chromosorb G(80—100 mesh). The working conditions were; column temperature 110°, injection temperature 140°, detector temperature 270°, sensitivity 3×10^{-10} afs, applied voltage 22 V. Several μl of a sample was injected into the gas chromatograph.

One of the gas chromatograms obtained is shown in Fig. 1. As is seen in Fig. 1, the peak of aluminum as its trifluoroacetylacetonate was clearly observed, and assigned from the comparison with the retention time of the peak of the standard aluminum trifluoroacetylacetonate, and the increase of the peak height of aluminum in the sample by addition of a definite amount of Al^{3+} to the digest solution before extraction with trifluoroacetylacetone–benzene.

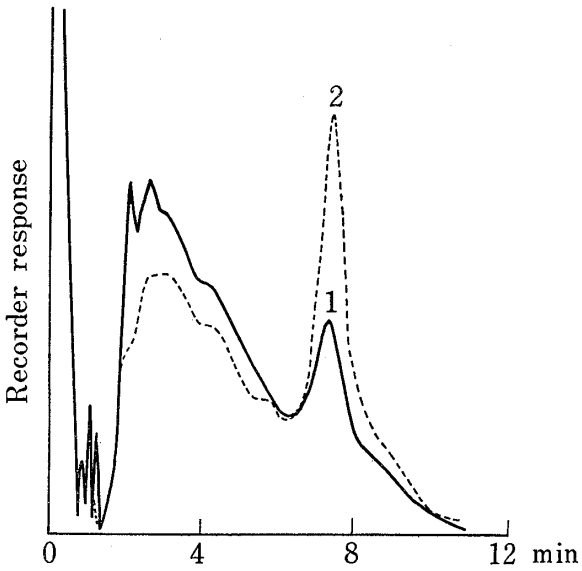

Fig. 1. Gas Chromatogram of Aluminum as Its Trifluoroacetylacetonate obtained from the Digest of Rat Liver

peak: 1 from the digest solution alone
2 after the addition of Al^{3+} to the digest solution

Moreover, the amount of aluminum in the sample size has been approximately estimated as a nanogram order and hence the total amount of aluminum in this rat liver may be considered in a microgram order.

The quantitation study of aluminum in biological materials by this method is being developed in our laboratory.

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