blood flow has been suggested to be propotional to the decrease in cardiac output in Rhesus monkeys.[15] In dogs, many investigators have reported that the cardiac circulatory function is significantly depressed by halothane inhalation.[16] However, the value of Vd may also be influenced by other factors, and further experiments are required.

Acknowledgement We are grateful to Dr. Hideya Saitoh, Department of Pharmacology, for valuable advice and for allowing us to use insuruments in his laboratory, and to Dr. Masahiro Nakano, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, for his helpful suggestions regarding pharmacokinetic analysis.

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[Chem. Pharm. Bull. 27(9)2220—2222(1979)]

UDC 547.742.04.08 : 543.544.45.061

Determination of Δ^1-Pyrroline as 2,3-Trimethylene-4-quinazolone

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(Received February 21, 1979)

2,3-Trimethylene-1,2-dihydroquinazolinium hydroxide, a reaction product of Δ^1-pyrroline and *o*-aminobenzaldehyde, was quantitatively converted to 2,3-trimethylene-4-quinazolone by chromic acid oxidation in dilute sulfuric acid. The reaction was successfully applied for determining as little as 10 nmol of Δ^1-pyrroline in deproteinized supernatant of rat liver by gas chromatography using a flame ionization detector.

Keywords——Δ^1-pyrroline; polyamine; γ-aminobutyraldehyde; 2,3-trimethylene-4-quinazolone; chromic acid oxidation; gas chromatography

In connection with the catabolism of naturally occurring diamines and polyamines, we have been interested in γ-aminobutyraldehyde, which cyclizes spontaneously to give Δ^1-pyrroline (I). The compound is produced by the action of either diamine oxidase on putrescine or spermidine oxidase on spermidine, although the latter activity has been found only in some bacteria.[2] The former activity is of interest in mammalian systems in view of the existence of an alternative pathway to γ-aminobutyric acid[3] and 2-pyrrolidone.[4,5] There is another report that I is the major natural co-substrate for thiaminase I.[6]

Before investigating the biological significance of I, we sought to establish a sensitive and reliable method for the determination of I in biological materials, since the only method available at present is based on a specific color reaction with *o*-aminobenzaldehyde (II), which has been applied to the determination of diamine oxidase activity[7] and to a specific enzymatic assay for spermidine.[2] The present paper describes the quantitative conversion of I to 2,3-trimethylene-4-quinazolone (V) as a procedure for the determination of I.

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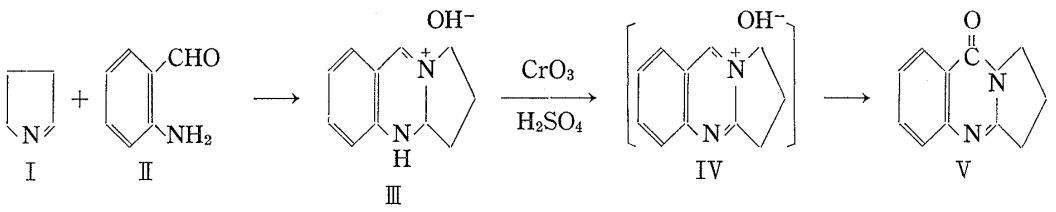

Chart 1

Chart 1 shows the color reaction of I with II in acidic or neutral medium to form 2,3-trimethylene-1,2-dihydroquinazolinium hydroxide (III). Schöpf and Oechler[8] obtained V as the picrate by adding picric acid to the colored solution that resulted from the reaction of I with II. We examined whether III could be rapidly and quantitatively oxidized to V with chromium trioxide in the presence of sulfuric acid. The time course of the oxidation reaction at room temperature was followed by thin-layer chromatography (TLC). The product III (*Rf* value, 0.27) completely disappeared aftet 15 min, and an ultraviolet absorbing spot of *Rf* 0.40 appeared. This spot disappeared, with the appearance of another ultraviolet absorbing spot of *Rf* 0.66, after 2 hr. These results strongly suggested that III was converted to the compound of *Rf* 0.66 through the intermediate of *Rf* 0.40. The compound of *Rf* 0.66 was purified by preparative TLC, and was identified as V by comparison with an authentic sample prepared according to the method reported previously.[9] The compound of *Rf* 0.40 did not crystallize, but the infrared spectral data of the extract from preparative TLC ($\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3400(OH), 1465(C=N)) suggested that it was 2,3-trimethylenequinazolinium hydroxide (IV), which has been obtained by dehydration of the picrate of III with lead tetraacetate in acetic acid.[8] The structural assignment is supported by the known transformation of 1,2-dihydro-4(3H)-hydroxyquinazolinium to 4(3H)-hydroxyquinazoline on mild oxidation.[10]

The conversion of I to V was next studied by gas chromatography with 2,3-tetramethylene-4-quinazolone as an internal standard. The time course of formation of V after the addition of chromium trioxide to the equilibrated color solution was examined. The formation of V reached a plateau after 2 hr, and V was not decomposed even after 3 days. Linearity of the peak height ratios was confirmed in the range of 10 nmol to 100 nmol of I per tube. A stoichiometric study using 1 μmol of I showed that the overall yield of V from I was 90% (Table I). Addition of I to deproteinized supernatant of rat liver gave similar good linearity (Fig. 1) and yield. Thus, the present conversion reaction should be useful

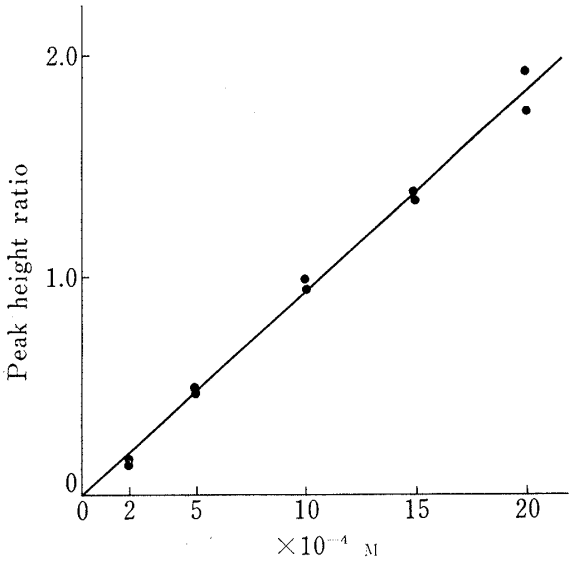

Fig. 1. Calibration Plot of I Added to Deproteinized Supernatant of Rat Liver

The supernatant of rat liver homogenized with two volumes of 0.5 N HCl and deproteinized with 0.2 volumes of 2 M perchloric acid, was used as a sample solution. First, 50 μl of 0.2—2 mM I and 0.1 ml of 50 mM II were added to 0.3 ml of the sample, then 50 μl of 1 mM internal standard and 0.3 ml of 0.64 M chromium trioxide in 0.8 M H_2SO_4 were added. Reaction times and gas chromatographic procedures were as described in "Experimental."

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Table I. Quantitative Conversion of I to V[a]

I added (μ mol)	Production of V[b] (μ mol) (mean±S.D.)	Yield (%)
1.00	0.90±0.04	90

a) Conditions were as described in "Experimental."
b) Five experiments were run.
V produced was gas-chromatographically determined from a calibration plot obtained with authentic V.

for the determination of I. The gas chromatographic method described here is roughly a hundred times more sensitive than the conventional colorimetry. The sensitivity could be further increased by combining the reaction with gas chromatography-mass spectrometry or immunoassay. An enzyme-immunoassay procedure for I is now being developed in this laboratory.

Experimental

Materials——I used in this experiment was obtained by dissolving γ-aminobutyraldehyde diethylacetal in 50 mM HCl; this compound was purchased from Aldrich Chemical Co., Inc., Milwaukee, and distilled before use. The purity was confirmed by elemental analysis. Chromium trioxide of the highest grade was from Koso Chemical Co., Ltd., Tokyo. II (mp 39—40°) was prepared by reducing *o*-nitrobenzaldehyde with $FeSO_4$,[11] and stored at −20° in ampoules under N_2. Authentic V or 2,3-tetramethylene-4-quinazolone used as an internal standard for gas chromatography was synthesized from α-pyrrolidone or α-piperidone and isatoic anhydride, respectively, by the published method.[9]

TLC——TLC was carried out on silica gel HF (E. Merck, Darmstadt, Germany) plates with a solvent system of BuOH–AcOH–H_2O (4:1:5 v/v upper phase).

Isolation and Identification of the Compound of *Rf* 0.66——Chromium trioxide (1.25 mmol) was added to the equilibrated color solution containing 40 μmol of **I** and 100 μmol of **II**. The final volume was 20 ml in 0.34 M H_2SO_4. The reaction mixture was kept at room temp. for 24 hr, and made alkaline with 5 N NaOH. The compound of *Rf* 0.66 produced was then extracted with ethylacetate, and purified by preparative TLC. Recrystallization from acetone gave colorless needles (4.5 mg, 61% as V), mp 110—111°. *Anal.* Calcd. for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.58; H, 5.39; N, 14.80. IR ν_{max}^{KBr} cm^{-1}: 1670, 1465.

Reaction Conditions for Conversion of I to V——Various concentrations (0.2—20 mM) of I in a volume of 1 ml were mixed with 1 ml of II (50 mM in 50 mM HCl). The color reaction reached a plateau after 2 hr at room temp. Then 1.5 ml of 0.64 M chromium trioxide dissolved in 0.8 M H_2SO_4 was added to the equilibrated color solution. The reaction mixture was kept at room temp. for 4 hr.

Determination of V by Gas Chromatography——The reaction mixture containing the internal standard was neutralized, made alkaline with 5 N NaOH, and extracted with an equal volume of benzene. Benzene was removed under an N_2 stream, then the residue was redissolved in ether. An aliquot of the ether solution was injected into a column (1 m × 4 mm I.D.) packed with a solid support, 80—100 mesh Shimalite W, coated with OV-17 (1.5%). Conditions for gas chromatography were as follows: column temp., 210° (injection port, 250°); flame-ionization detector oven temp., 250°; carrier gas(N_2) flow rate, 30 ml/min. Retention times for V and the internal standard were 5 and 7 min, respectively.

Acknowledgement The authors wish to thank Dr. Masashi Okada, Director of this institute, for his support during this work.

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