log P=2.35 for *o*-tolidine in the *n*-octanol-water system. The log P value of *o*-toluidine was reported as 1.29.[21] Thus, the log P value of *o*-tolidine can be estimated as 2.58 (twice 1.29) or 2.19 (twice 1.29 minus twice 0.193).[22] These calculated values are reasonable in magnitude compared with that obtained here. In conclusion, it appears that the true partition coefficient, pK_{a1}, and pK_{a2} of 6059S or *o*-tolidine can be evaluated in terms of Eqs. 1 and 2 under the assumptions mentioned in the text.

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22) The log P values of organic substances can be calculated by using the sum rule of such hydrophobic fragmental constants as π or f. The π[23] and f[21] values of the hydrogen atom are 0.00 and 0.193, respectively.
23) C. Hansch, A. Leo, S.H. Unger, K.-H. Kim, D. Nikaitani, and E.J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

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Spectrophotometric Determination of Bromazepam[1]

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Bromazepam (7-bromo-1, 3-dihydro-5-(2-pyridyl)-2H-1, 4-benzodiazepin-2-one) was determined by formation the chelate compound with iron(II) followed by extraction from the organic phase into an aqueous solution. The absorption spectrum of the chelate compound in aqueous solution showed maximum absorbance at 580 nm and the absorbance was stable for about 5 hr at room temperature.

The calibration curve was linear from 0.2 to 50 μg/ml of bromazepam and the sensitivity of the procedure was 0.2 μg/ml of bromazepam.

This procedure was applied to the determination of bromazepam in rat blood. The recovery of added bromazepam from rat blood ranged from 96 to 100.5%, with a mean recovery of 99.1%.

Keywords——bromazepam; 1,4-benzodiazepine derivatives; iron(II) chelate compound; spectrophotometric determination; thin-layer chromatography; rat blood

Introduction

Bromazepam[3] was synthesized by Fryer *et al.*[4] and has been clinically evaluated for the control of the obsessive compulsive neurosis.[5] Bromazepam can be hydrolyzed with strong mineral acid or decomposed thermally to yield an aromatic primary amine which can be determined by the Bratton–Marshall procedure[6] or by gas–liquid chromatography.[7]

Sabation *et al.*[8] reported that ferrous ions formed purple complexes with pyridyl benzodiazepin-2-ones; these compounds have a basic dipyridyl type bond structure which has excellent

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metal complexing properties. The composition of the complex was 3: 1 [pyridyl benzodiazepin-2-one: iron(II)]. This paper presents a procedure for the spectrophotometric determination of bromazepam itself.

Experimental

Materials

1 M Acetate Buffer (pH 4.5)——Glacial acetic acid (60 g) was added to a solution of 82.0 g of sodium acetate in 800 ml of deionized water, and the solution was diluted to 1 liter with deionized water.

Stock Iron Solution——Pure iron wire (E. Merck, Darmstadt, Germany) (0.100 g) was dissolved in 50 ml of boiling conc. nitric acid. The solution was cooled and diluted to 100 ml with deionized water.

Color Reagent——Three grams of L-ascorbic acid (Special grade, Wako Pure Chemical Industrial Co., Tokyo) and 0.5 ml of the stock iron solution were added to 80 ml of acetate buffer (pH 4.5) and the solution was diluted to 100 ml with the buffer. Next, 0.05 ml of 0.02% Sterox S.E. (Hartman–Redden Co., Philadelphia, Pa.) was added and the solution was kept in a refrigerator.

Bromazepam——The materials was recrystallized from legroin (90—110°), mp 246—248°. It was practically insoluble in water but is soluble in organic solvents, *i.e.* ethyl alcohol, ethyl acetate and chloroform.

2-Amino-5-bromobenzoyl Pyridine (ABBP)——ABBP was obtained by acid hydrolysis of bromazepam and recrystallized from legroin (90—110°), mp 96—98°.

Thin layer chromatography was carried out on plates precoated with silica gel GF254 from E. Merck, 61 Darmstadt, Germany and from Yazawa Kagaku Instr. Co., Tokyo, Japan.

Developing Solvents——Solvent I: ethyl acetate: ether (4: 1 v/v). Solvent II: chloroform: methanol (2: 1 v/v).

Spectrophotometery was carried out with a Hitachi model 101 spectrometer (Hitachi Co., Japan).

Animals——Young albino rats of the Wistar strain were used as test animals (male; 4 weeks old).

Standard Procedure

Ten grams of authentic bromazepam was dissolved in 100 ml of ethyl acetate of prepare a standard solution. Working solutions were prepared by diluting the standard solution with ethyl acetate. Each 1.0 ml of working solution contained 0.2 to 20 μg of bromazepam. Five milliliters of the color reagent was added to 1.0 ml of the working solution with vigorous mixing and the whole was allowed to stand for 3 min. The resulting brilliant violet color in the aqueous portion was allowed to develop for 5 min at room temperature and its absorbance measured at 580 nm against a reagent blank. A reagent blank was prepared by carrying 1 ml of pure ethyl acetate through the above procedure.

Extraction of Bromazepam in Blood——Bromazepam was suspended in 3% arabic gum solution and rats were gave 100 mg of the drug per kilogram body weight orally, as a single dose, in the form of this suspension. Rat blood collected at 1 hr after administration of the drug was deproteinized with acetic acid. The resulting solution was adjusted to pH 8.0 with Na_2CO_3, extracted with about 5 volumes of ethyl acetate and assayed spectrometrically by means of the chelate reaction in acetate buffer (it was diluted to 10 ml with deionized water before measuring the absorbance). The drug content corresponding to this absorbance was read off from a standard calibration curve.

Results and Discussion

Absorption Spectrum

The absorption spectrum of the colored compound in aqueous solution, pH 4.5 (Fig. 1), showed a maximum at 580 nm.

Standard Calibration Curve

A typical calibration curve is shown in Fig. 2. The calibration curve was linear from 0.2 to 50 μg/ml of bromazepam.

Color Stability

As shown in Fig. 3, the maximum absorption of the colored compound was stable for about 5 hr at room temperature (23±2°).

Effect of Copper (II) Ions on the Color Reaction between Bromazepam and Iron (II)

In order to examine the effects of copper on the reaction, 10 to 500 μg/ml of copper (II) was added to the color reaction system and the mixture was allowed to stand for 3 to 5 min.

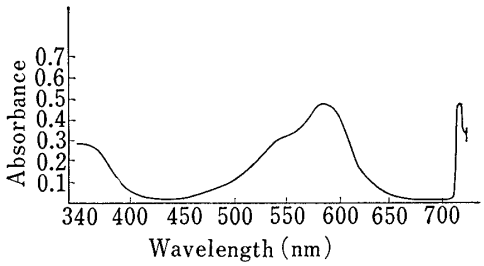

Fig. 1. Absorption Spectrum of the Colored Solution

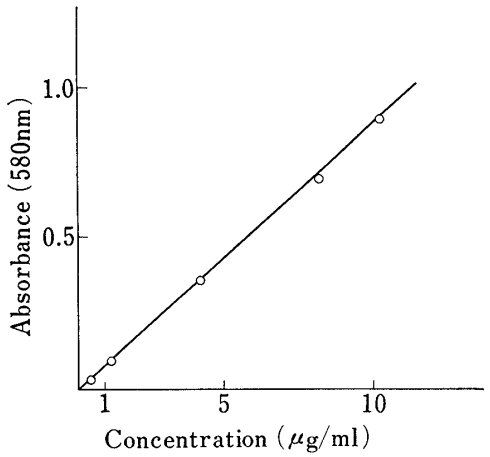

Fig. 2. Calibration Curve for Bromazepam

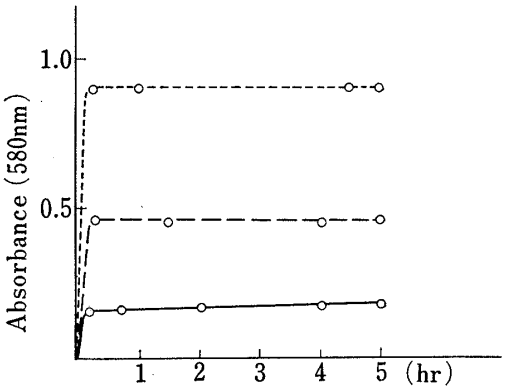

Fig. 3. Stability of the Colored Solution

○-----○ 10 μg/ml, ○-----○ 5 μg/ml, ○——○ 2 μg/ml.

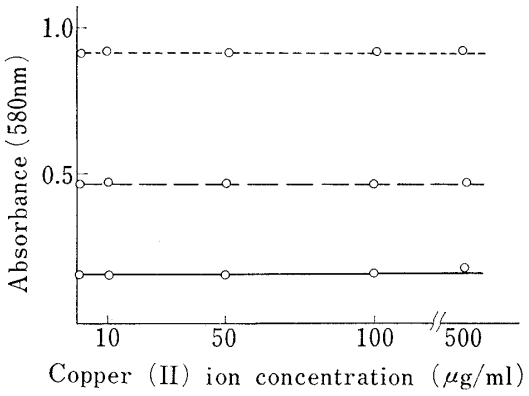

Fig. 4. Effect of Copper Ions on the Color Reaction between Bromazepam and Iron

○-----○ 10 μg/ml, ○-----○ 5 μg/ml, ○——○ 2 μg/ml.

Table I. *Rf* Values and Color Reaction of Bromazepam and Its Decomposition Product

Substance	Solvent I *Rf* value	Solvent II *Rf* value	Cupric nitrate solution(pH 4.5)	Color reagent(pH 4.5)
Bromazepam	0.35	0.70	Green	Violet
Decomposition product	0.70	0.85	Dark green	—
ABBP	0.70	0.85	Dark green	—

Solvent I: Ethyl acetate/Ether (4: 1),.
Solvent II: Chloroform/Methanol (2: 1).
Adsorbent: Silica-Gel G (Merck).
Thickness: 250 μ.

As shown in Fig. 4, no interference with the color reaction was observed.

Rf values and Color Reactions of Bromazepam and Its Decomposition Product

About 30 μl of a mixture of bromazepam and its decomposition products, obtained by acid hydrolysis, was applied as a horizontal line to a silica gel plate and developed with developing solvent I or II. The plate was sprayed with the color reagent. As shown in Table I, the *Rf* values of the colored spots were recorded.

Table II. Recovery of Bromazepam from Rat Blood

Sample	Bromazepam added (μg/ml)	Bromazepam recovered (μg/ml)	Recovery (%)
1	2	2.01	100.5
2	5	4.82	96.5
3	10	10.01	100.1
4	20	19.98	99.1
Mean			99.0
Whole blood[a)]	100 (mg/kg)	14.5+37[b)] (μg/ml)	

a) Blood level in rats 1 hr after oral administration of 100 mg/kg of bromazepam.
b) Mean ±SD. (10 rats).

Recovery of Bromazepam from Rat Blood

One milliliter of bromazepam sample (2 to 20 μg/ml) in 3% arabic gum solution was added to 2 ml of whole blood. After deproteinization, the solution was adjusted to pH 8.0 with Na_2CO_3 and assayed spectrometrically in accordance with the standard procedure. Recovery of added bromazepam ranged from 96 to 100.5% with a mean recovery of 99.18%. Bromazepam was also administered orally to rats, and the blood level of bromazepam determined at 1 hr after administration is shown Table II. The present procedure, utilizing a specific reaction for the determination of intact bromazepam, is suitable for identifying and quantitating bromazepam in prepared tablets and in biological fluids.

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Adsorption of Styrene on Activated Carbon and Regeneration of Spent Activated Carbon

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Adsorption of styrene on activated carbon was investigated on the basis of the adsorption isotherm, isosteric heat of adsorption, and compressed volume of condensed styrene. Regeneration of spent activated carbon was examined by the extraction of styrene with organic solvents. The Langmuir equation could be applied to the adsorption isotherms of styrene on activated carbon. The isosteric heats of adsorption of styrene on activated carbon Nos. 1—3 and 5 were less than twice the value of the heat of condensation of styrene (ΔH_0=9.64 kcal/mol) in the range of θ 0.2 to 1.0, and therefore, styrene adsorbed in this range of θ seemed to be physisorbed on activated carbon. Styrene physisorbed on activated carbon seemed to be compressed in the pores. Spent activated carbon could be regenerated by extracting the styrene with acetone at about 30° and its adsorptive capacity became approximately equal to that of fresh activated carbon.

Keywords——styrene; activated carbon; adsorption; isosteric heat of adsorption; compression; regeneration

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