The Micro Determination of Isoniazid by *N*-Bromosuccinimide

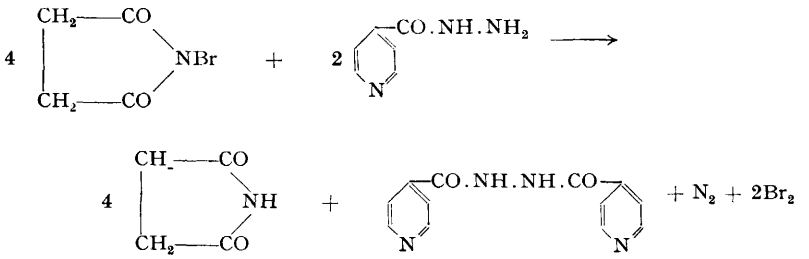
By M. Z. BARAKAT and MONIER SHAKER

(*Biochemistry Department, Faculty of Veterinary Medicine, Cairo University, Giza, Cairo, Egypt*)

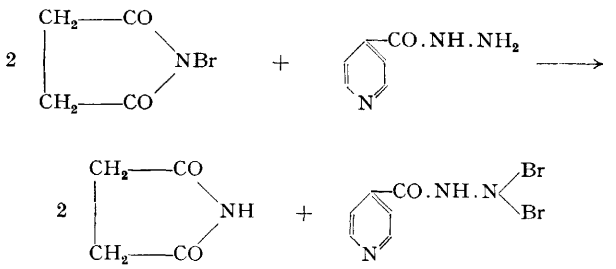
In the presence of dilute hydrochloric acid, *N*-bromosuccinimide readily and quantitatively oxidises aqueous solutions of isoniazid. The reaction takes place at room temperature and *N*-bromosuccinimide is irreversibly reduced to succinimide. The mechanism of the reaction is discussed. The micro determination of isoniazid itself, and in certain pharmaceutical preparations with standard *N*-bromosuccinimide solution is described. The experimental error does not exceed ± 2 per cent.

Assay of isoniazid, according to the British Pharmacopoeia 1958, involves the use of 0·1 N bromine. The micro determination of hydrazine salts and certain derivatives by the use of *N*-bromosuccinimide has recently been reported[1].

There appears to be no report in the literature concerning the reaction between *N*-bromosuccinimide and isoniazid. The action of *N*-bromosuccinimide on isoniazid in macro quantities in hot glacial acetic acid is reported. The reaction is analogous to the action of *N*-bromosuccinimide on phenylhydrazine[2] and proceeds as follows—



This reaction has been applied to the micro determination of isoniazid in aqueous acid medium at room temperature, with methyl red as an indicator.[1] During the titration process no free bromine is liberated, and the reaction is quantitative according to the following equation—



Experimental

Action of *N*-bromosuccinimide on isoniazid—

A 1·375-g portion of isoniazid (0·01 mole) was dissolved in 20 ml of glacial acetic acid, and 3·56 g of *N*-bromosuccinimide (0·02 mole) were dissolved in 30 ml of hot glacial acetic acid. The two solutions were mixed together and heated on a water-bath for 30 minutes. Strong effervescence was observed owing to evolution of nitrogen gas. A heavy yellowish precipitate was also formed which was filtered off, giving solid A and filtrate B. Solid A was washed with glacial acetic acid and left to dry. This solid was then dissolved in the least amount of cold water (5 ml) and 2 per cent. sodium hydroxide solution was added dropwise until a pH of 5 was obtained and a colourless crystalline compound was deposited. Excess of sodium hydroxide solution would dissolve the deposited solid. The latter was filtered off, washed with water and recrystallised from hot

water giving 0·6 g of 1 : 2-diisonicotinoyl hydrazine,[3] which was identified by its melting-point of 255° to 260° C (decomp.), and mixed melting-point determinations with an authentic specimen that showed no depression. Filtrate B was distilled *in vacuo* and the solid residue was crystallised from benzene giving 0·9 g of succinimide, identified by its melting-point of 124° to 125° C and its mixed melting-point determinations with an authentic sample that showed no depression.

VALIDITY OF THE REACTION FOR QUANTITATIVE DETERMINATION—

Before applying the reaction to the determination of isoniazid in test solutions, the authors decided to verify quantitatively the reaction between *N*-bromosuccinimide (0·178 per cent.; 1 millimole in 100 ml) and isonicotinohydrazide (0·1371 per cent.; 1 millimole in 100 ml) following the previously reported procedure.[1] A similar series of experiments was carried out with *N*-bromosuccinimide solution containing twice the number of molecules of solute as in the first solution. It was found that the reaction was stoicheiometric in the presence of dilute hydrochloric acid (5 per cent. v/v) at room temperature. The results were—

Volume of isoniazid solution (1 mmole per 100 ml), ml ..	5	4	3	2	1
Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml	10	8·05	6·02	4·02	2
Titre of *N*-bromosuccinimide solution (2 mmole per 100 ml), ml	5·01	4·03	3·0	2·0	1·01

METHOD

REAGENTS—

Methyl red solution, 0·04 per cent. w/v in 95 per cent. ethanol.
Dilute hydrochloric acid, 5 per cent. v/v.
N-*Bromosuccinimide, 0·1 per cent. w/v, aqueous.*

PROCEDURE—

Place an accurately measured volume, *e.g.*, 5 ml, of the isoniazid solution in a 50-ml conical flask. Add an equal volume of dilute hydrochloric acid (5 per cent. v/v) and 2 drops of methyl red indicator solution. Titrate the mixture with 0·1 per cent. w/v *N*-bromosuccinimide, added dropwise, with continuous shaking of the flask from a microburette graduated at 0·01-ml intervals. The end-point is reached when the last drop of titrant discharges the red colour. A blank experiment is carried out simultaneously and the reading is subtracted from the titre before calculation.

Calculate the isoniazid content of the sample solution from the expression—

$$\text{Isoniazid present in mg or } \mu g = V \times C \times \frac{137{\cdot}1}{356}$$

Where V is the titre of *N*-bromosuccinimide solution in millilitres and C is the concentration of *N*-bromosuccinimide in mg or μg per ml.

APPLICATION OF THE METHOD

DETERMINATION OF ISONIAZID—

The proposed method was applied to a range of 1 to 10-ml solutions of 0·1 and 0·01 per cent. isoniazid with 0·1 and 0·05 per cent. of *N*-bromosuccinimide solution, and with drug recoveries ranging from 99·4 to 100·6 and 98·7 to 101 per cent. of theory, respectively.

1 ml of 0·1 per cent. of *N*-bromosuccinimide solution ≡ 0·3851 mg of isoniazid.
1 ml of 0·05 per cent. of *N*-bromosuccinimide solution ≡ 192·55 μg of isoniazid.

ASSAY OF ISONIAZID TABLETS AND INJECTIONS—

When the method was applied to a sample of tablets containing 50 mg of isoniazid and to a sample of isoniazid injection, satisfactory recoveries were obtained.

The results show that the proposed method is accurate to determine concentrations as low as 0·1 mg of isoniazid, while the British Pharmacopoeia 1958 method[4] recommends the use of at least 40 mg of isoniazid.

REFERENCES

1. Barakat, M. Z., and Shaker, M., *Analyst*, 1963, **88**, 59.
2. Barakat, M. Z., Abd El-Wahab, M. F., and El Sadr, M. M., *J. Amer. Chem. Soc.*, 1955, **77**, 1670.
3. Albert, A., and Ress, C. W., *Biochem. J.*, 1955, **61**, 128.
4. British Pharmacopoeia, 1958, p. 355.

Received *April 12th*, 1965