

On the Occurrence of Isoguanine in Pig Blood

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2-Hydroxy-6-aminopurine (isoguanine, oxyadenine) was first synthesized by Emil Fischer in 1897¹.

The first report on its occurrence in nature appeared in 1927 when Buell and Perkins² claimed to have isolated isoguanine from pig blood in a yield of about 5 mg per liter.

In 1932 Cherbuliez and Bernhard³ isolated a nucleoside from croton bean (*Croton tiglium* L.) and identified the aglycone as isoguanine and the sugar as D-ribose by the properties of various derivatives. The sugar was then crystallized by Spies and Drake⁴, and Spies⁵ confirmed that the purine in this nucleoside was isoguanine by converting it to xanthine.

Isoguanine has further been identified by Purrman¹² among the nitrogenous bases in wings of certain butterflies (*Catopsilia*).

Schütz⁷ has reported that he was unable to isolate any isoguanine from pig blood with the procedure of Buell and Perkins². The only purine found was adenine. He also questioned the work of Cherbuliez and Bernhard as to the identity of the purine in the crotonoside but later work has proved these doubts to be unfounded.

The confirmation of the occurrence of isoguanine in an animal organism would be of interest in connection with the transformation of adenine into guanine demonstrated to occur in rat by Brown, Roll and Plentl⁷ with the aid of isotopically marked purines. We have therefore reinvestigated the composition of the purine fraction in pig blood.

It was found that partition chromatography on starch, as developed for purines by Edman, Hammarsten, Löw and Reichard⁹, can be used to separate isoguanine from its mixture with adenine and guanine as exemplified in Fig. 1.

Recently Bendich, Tinker and Brown¹⁰ have described a method to purify and characterize the natural product from croton bean with countercurrent distribution and they have also worked out a new synthesis of isoguanine.

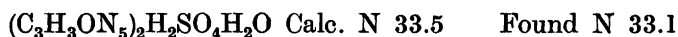
Following the procedure of Buell and Perkins ² for the preparation of isoguanine from pig blood we have tried to demonstrate the presence of isoguanine in the later stages of their procedure by means of chromatography on starch. With this sensitive method no trace of isoguanine could be demonstrated. The ultimate product was practically pure adenine.

This is in accordance with the findings of Schütz ⁶ and taken together with the note in the paper by Mitchell and Houlahan ¹¹ it appears improbable that isoguanine has ever been isolated from higher animals.

EXPERIMENTAL

Isoguanine

Isoguanine, prepared from the crotonoside according to Cherbuliez and Bernhard ³, was first used as a reference compound. We are greatly indebted to professor Bernhard for a sample of the crotonoside. After hydrolysis of the natural crotonoside and one recrystallization from sulfuric acid pure isoguanine sulfate was obtained.



Later a sample of synthetic isoguanine ¹⁰ was kindly furnished us by Dr. Bendich. The two samples had identical absorption curves (Fig. 3) and also gave identical x-ray powder diffraction patterns. We are greatly indebted to Dr. Einar Stenhagen, Upsala, for the latter measurements.

Preparation of purines from pig blood

For the preparation of the purine from pig blood the procedure of Buell and Perkins ² was followed. In two different preparations from 5 l of pig blood the yields of purine in the final step were 15 and 60 mg.

In order to separate isoguanine from other purines expected to contaminate the preparation, starch chromatography was employed. The technic was essentially that of Edman ⁸ and Edman, Hammarsten, Löw and Reichard ⁹. The starch was freed from soluble material absorbing in the ultraviolet through extraction with 80 per cent dioxane-water for 24 hours in a Soxhlet apparatus. Likewise methyl glycol and *n*-butanol were freed from most of the absorbing material by distillation through a Widmer column.

As a model experiment a mixture of adenine, guanine and isoguanine was separated on the starch column. 1.98 mg adenine, 2.05 mg guanine and 1.37

mg purine

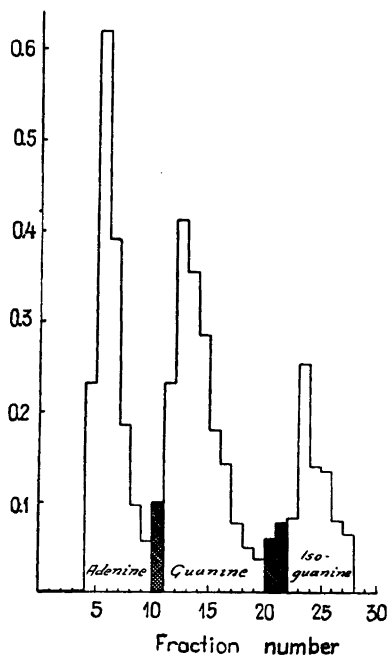


Fig. 1. Chromatography on starch of mixture containing adenine, guanine and isoguanine. Shaded areas indicate mixed fractions.

mg purine

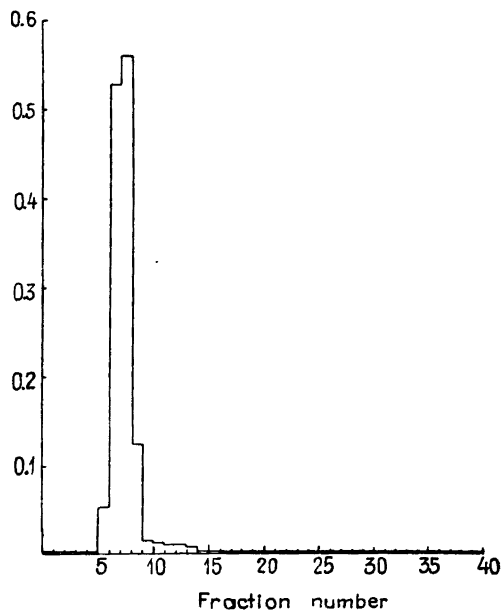


Fig. 2. Chromatography on starch of purine from swine blood.

mg isoguanine were dissolved by heating on the water bath in 0.7 ml methyl glycol and 0.1 ml *N* sodium hydroxide. To this solution was added 9.8 ml *n*-butanol-water (87 % v/v *n*-butanol) and the whole transferred to the top of the starch column. The height of the column was 205 mm and the diameter 24 mm. The chromatogram was developed in a mixture of *n*-butanol /water/ methyl glycol in the proportions 12 : 2 : 1 by volume. The effluents were cut with an interval of one hour, corresponding in this experiment to a fraction volume of about 12 ml. After completion of the chromatography all fractions were evaporated to dryness *in vacuo*, subsequently dissolved in 1 *N* hydrochloric acid and the absorption measured at wave lengths 248, 262 and 285 μ . From the extinctions at these wave lengths coefficients can be formed suitable for the identification of the individual purines. The result of the fractionation can be seen in Fig. 1.

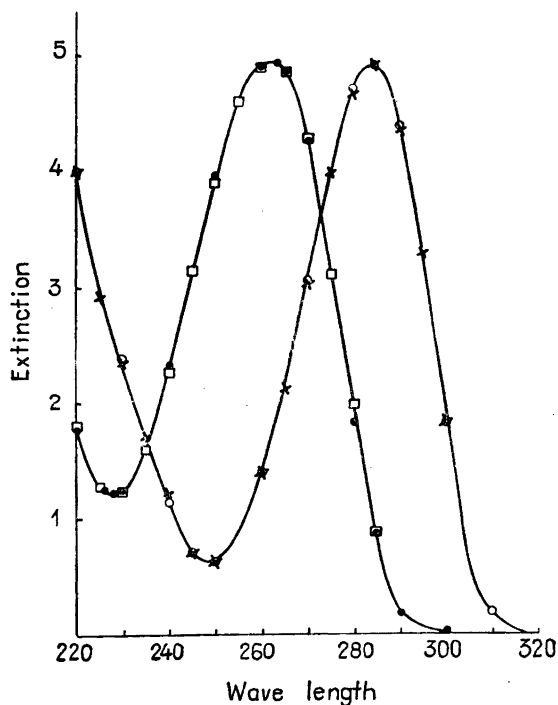


Fig. 3. Ultraviolet absorption curves of a) adenine (Hoffmann-La Roche), $5.6 \cdot 10^{-3}\%$, $-\square-\square-$; b) chromatography fraction no. 7, $-\bullet-\bullet-$; c) isoguanine (synthetic), $6.0 \cdot 10^{-3}\%$, $-\circ-\circ-$; d) isoguanine (from crotonoside), $6.1 \cdot 10^{-3}\%$, $-x-x-$. Solvent *N HCl*, $d = 1$ cm.

Under identical conditions a fractionation was carried out on a 2 mg specimen of purine (as hydrochloride) from pig blood. The result is presented in Fig. 2. This experiment was repeated on an independent preparation with the same result. The complete ultraviolet absorption curve of fraction no. 7 was measured and this is compared in Fig. 3 with the corresponding curves for pure adenine (Hoffmann-La Roche) and samples of synthetic and natural isoguanine.

Since it were conceivable that the isoguanine had been lost during the later stages of the purification procedure, it was also tested for isoguanine at an earlier stage. After the precipitation with cupric sulfate and sodium bisulfite the preparation was freed from copper with hydrogen sulfide in hydrochloric acid. The filtrate was evaporated to dryness and a sample was taken for chromatography. The chromatogram showed only one peak. This appeared on the place of adenine and had the quotient $\frac{E_{262}}{E_{248}} = 1.37$ characteristic of adenine.

The x-ray diffraction pattern of the product isolated from blood was identical with that of adenine hydrochloride.

SUMMARY

1. A chromatographic procedure is described whereby small amounts of isoguanine, adenine and guanine can be separated.
2. Using this technic isoguanine could not be found in pig blood.

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LITERATURE

1. Fischer, E. *Ber.* **30** (1897) 2226.
2. Buell, M. V., and Perkins, M. E. *J. Biol. Chem.* **72** (1927) 745.
3. Cherbuliez, E., and Bernhard, K. *Helv. Chim. Acta* **15** (1932) 164, 978.
4. Spies, J. R., and Drake, N. L. *J. Am. Chem. Soc.* **57** (1935) 774.
5. Spies, J. R. *J. Am. Chem. Soc.* **61** (1939) 350.
6. Schütz, F. A. *Biochem. Z.* **273** (1934) 52.
7. Brown, G. B., Roll, P. H. and Plentl, A. A. *Federation Proc.* **6** (1947) 517.
8. Edman, P. *Acta Chem. Scand.* **2** (1948) 592.
9. Edman, P., Hammarsten, E., Löw, B., and Reichard, P. *J. Biol. Chem.* **178** (1949) 395.
10. Bendich, A., Tinker, J. F., and Brown, G. B. *J. Am. Chem. Soc.* **70** (1948) 3109.
11. Mitchell, H. K., and Houlahan, M. B. *Federation Proc.* **5** (1946) 370.
12. Purrrman, R. *Ann.* **544** (1940) 182.

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