

Was Citrulline First a Laxative Substance?: The Truth about Modern Citrulline and Its Isolation

Konstantinos C. FRAGKOS, Alastair FORBES

Centre for Gastroenterology & Nutrition, Division of Medicine, University College London

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Abstract: Citrulline is a non-protein amino acid and is produced by the enterocytes of the small bowel. The isolation though of citrulline is generally ascribed to the 1930s. In the present article, we demonstrated that before 1930, there was use of the term citrulline, signifying a resin produced by *Citrullus Colocynthis*. This citrulline is different from modern citrulline. However, neither was modern citrulline isolated in 1930 but somewhat earlier. Reviewing the original manuscripts, Koga and Ohtake (1914) did indeed isolate citrulline for the first time and at least half a dozen other researchers cite their work. Even though their work didn't lead to the determination of the structure and nature of citrulline, theirs was the first to isolate it. Our results have a certain historical and scientific significance and are discussed in extent.

Key words: citrulline, isolation, history, laxative

Introduction: Intestinal Failure and Citrulline

Intestinal failure (IF) occurs when there is reduced intestinal absorption so that macronutrient and water and electrolyte supplements are needed to maintain health or growth. Nutrient/fluid requirements determine whether IF is termed severe, moderate, or mild. Severe is when parenteral, moderate when enteral, and mild when oral nutritional fluid supplements are needed.^{1,2)} The most common cause of IF is short bowel syndrome. Normal human small intestinal length, measured from the duodenojejunal flexure at autopsy or surgery, varies from about 275 cm to 850 cm, and tends to be shorter in women. After intestinal resection it is important to refer to the remaining length of small intestine measured at surgery or with an opisometer.²⁾ In general, nutritional/fluid supplements are likely to be needed if less than 200 cm of small bowel remains. The most common reasons for a short bowel in adults are Crohn's disease, superior mesenteric artery thrombosis, and irradiation damage.¹⁾

Citrulline's unique metabolism which involves production at the intestinal enterocyte has prompted suggestions that plasma citrulline level could be a reliable marker of gut function.³⁾ This led to a hypothesis that citrulline may be a 'conditionally' essential amino acid in short bowel syndrome, even if it is not incorporated into proteins. Healthy patients with normal intestinal mucosa function and normal renal function have a citrulline level between 30 and 50 $\mu\text{mol/l}$ with a median of 40 $\mu\text{mol/l}$.^{4,5)} Although this range for plasma citrulline levels mainly comes from studies in Western Europe and North America, a Chinese study on 33 healthy Chinese subjects found a mean plasma citrulline level of $16.87 \pm 5.97 \mu\text{mol/L}$ (range 19–54) measured with HPLC.⁶⁾ This finding is very interesting, because it creates a paradox in the multiple findings of the high diagnostic accuracy of citrulline level at 20 $\mu\text{mol/L}$.

Many researchers have examined whether citrulline levels are a marker of enterocyte mass or absorptive

function. Their study groups included patients with short bowel syndrome, celiac disease, Crohn's disease, patients who are on anti-neoplastic treatment or who have undergone intestinal transplantation. A recent meta-analysis of ours showed that the pooled correlation coefficient of citrulline levels with small bowel length in SBS patients was 0.697 while the correlation coefficient of citrulline levels with absorptive test was lower (0.326).⁷⁾

Historical Fallacies

“Citrulline was first isolated in 1930”.⁸⁾ This is a common phrase in the nutritional and gastroenterology literature regarding citrulline. Nevertheless, some interesting historical sources detail that the amino acid citrulline was discovered much earlier than that, while there was use of another citrulline at the late 19th century.

In the *North Carolina Medical Journal*, January 1883,⁹⁾ we read the following passage:

“The colocynthus purum prepared by Merck [...] produces watery stools with moderate tormina¹⁰⁾ [...] There is also *a resinoid substance called citrullin*, extracted from the colocynthus fruit, insoluble in water, which, when taken internally in the dose of 5 milligrammes to 1 centigramme, or if administered hypodermically in the same dose [...] will produce the desired effect”. (ref. 9, p.44)

Similar excerpts appeared then in other journals, such as the *Medical Age*¹¹⁾ (Figure 1) and the *Journal of the American Medical Association*¹²⁾, while even a significant gastroenterologist of that time, Ismar Boas (1858–1938),¹³⁾ comments on citrulline's usefulness with difficult cases of constipation: “Laxatives may often be administered by the rectum in the form of small enemata [...] The most serviceable [...] for especially obstinate cases, are colocynthin and *citrullin*”.¹⁴⁾ To the reader of nutrition and gastroenterological articles, this early mentioning of the word citrullin[e] causes some surprise. That is because citrulline is

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GLYCERIN AND CITRULLIN SUPPOSITORIES.

Suppositories, as a rule, are ineffective where constipation is due to febrile diseases, to affections of the brain and spinal cord, or to mechanical obstruction of the intestinal circulation. In such cases, however, the same suppositories, fortified by the addition of *Citrullin*, will secure in most satisfactory manner the desired result; the latter are

Figure 1. Passage from *The Medical Age* 1894; 12: 115, mentioning citrullin[e] suppositories

generally regarded to have been first discovered in the 1930s¹⁵⁾ and is part of the urea cycle taking place mainly in the liver; citrulline's pharmacological action has not yet been studied in humans, so this laxative effect also amazes us.

The purpose of this article is to answer whether citrulline had been discovered before the 1930s and much more whether it had been utilized therapeutically in some way we lack knowledge of - even today.

Early sources mentioning the term “citrulline”

We performed a systematic review of all texts before 1930, which mentioned the term “citrulline”. A preliminary analysis had shown us that the synonym terms “citrullin” and “citrullinum” were also in use. Thus, we searched databases with extensive digitized volumes of rare books: Google Books (<http://books.google.com>), Internet Archive (<http://www.archive.org>), and Gallica (<http://gallica.bnf.fr>). These three databases are the largest online sources of mass digitization projects of books from a large range of American and English universities, while Gallica is a large-scale digitization program of the National Library of France. We used the search term “citrulline or citrullin or citrullinum” and time limit from 1800–1930. A source of bias might have been introduced, due to the fact that languages which do not use the Latin alphabet were not searched.

Our results revealed that 170 books and journal volumes have the word citrulline among their pages. This figure might increase to 254 volumes, if other editions are considered as well. Specifically, these were all published between 1882 and 1930 and include in their majority English, French, and German texts, while very few were in Dutch, Swedish, and Italian. A full account of all texts is given in Appendix A. All texts mention the results of two main articles, written by Hiller¹⁶⁾ and Kohlstock,¹⁷⁾ discussing subcutaneous and rectal injection of purgatives.

Is this citrulline, the modern citrulline?

This citrulline is a resin produced by the pulp of *Citrullus colocynthis*. *C. colocynthis* Schrad (family Cucurbitaceae), also known as colocynth or bitter apple, is a common weed found in countries of the Middle East and the Mediterranean (Figure 2). *C. colocynthis* has been used medicinally since ancient times. The fruits and seeds are used as purgative and have been suggested to possess antitumour activity.¹⁸⁾ From the pulp of *C. colocynthis*, α -elaterin, α -elaterin-2-D-glucopyranoside, citrullol, and an alkaloid with strong purgative action have been isolated,¹⁹⁾ while the other parts of this weed contain several more substances.²⁰⁾ When overconsumed, it can cause colitis.²¹⁾ This citrulline is different from the modern citrulline, an amino acid which participates in the urea cycle.⁵⁾

So, the term citrulline had been in use for about 40 years from 1882 reaching the early 1920s, referring to a strong purgative used occasionally in humans. This discussion was stimulated by two articles dealing with subcutaneous and rectal injection of purgatives.^{16,17)} In summary, both authors found that citrulline could be administered subcutaneously or rectally (dose: 5 mg to 20 mg) in cases of chronic constipation, but it was followed by adverse effects of severe pain, oedema and redness of the skin. Rectal injections were tolerated more easily than subcutaneous ones. Its use was most possibly hindered because of a high price or low availability,²²⁾ while it was used more frequently in veterinary medicine.²³⁾ It must have passed into oblivion due to the infrequent use and the ineffectiveness it presented as a laxative. A possibility is



Figure 2. A drawing of *C. colocynthis* from D.M.R. Culbreth. *A Manual of Materia Medica and Pharmacology*. 6th ed. Philadelphia: Lea & Febiger; 1917. p. 585

that it could have changed name over time, making it difficult to track in scientific literature.

The history of modern citrulline's isolation

Modern citrulline is a non standard amino acid and in humans its plasma content is derived largely from the amount produced in enterocytes of the small bowel (Figure 3).²⁴⁾ Citrulline's first isolation from the juice of the watermelon (*Citrullus vulgaris Schrad*) has generally been attributed to Mitsunori Wada,^{5,8,25,26)} who isolated citrulline and determined its chemical formula in 1930, naming the substance he isolated *citrulline*.¹⁵⁾ We would like to add further value to current literature on citrulline, by mentioning that it was isolated earlier than 1930, and specifically in 1914. We present the supporting evidence.

The first isolation of citrulline occurred in 1914 by Yotaro Koga and Ryo Ohtake,²⁷⁾ who isolated citrulline from the juice of the watermelon and said that a substance was present in it with the chemical formula $C_6H_{13}N_3O_3$ (Figure 4). They didn't elucidate though the structure of this new substance nor did they name it. A possible inhibiting effect of this understanding may be the fact that it was written in Japanese, making it difficult for European and American scientists to grasp the significance of this paper. Although Japanese people had begun studying European languages from the early 19th Century, this was not the case with Europeans, whose knowledge of Japanese was rather scarce.²⁸⁾ We have attached a translation of their article to Appendix B.

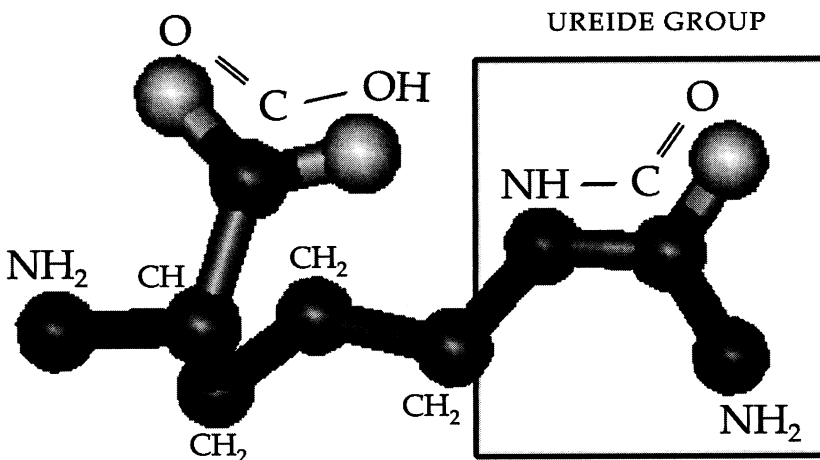


Figure 3. Citrulline structure. In a box is the characteristic group of citrulline, the ureide group (NH₂-CO-NH)

文 報

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五一九

西瓜搾汁の成分研究報告

農學士 古賀彌太郎
大 塚 丁

成熟せる西瓜 (*Citrus vulgaris* var. *salicifolia*) 二十三個(重量一八〇)を採り赤色瓜内部を別ち壓搾して其汁液を集め濾紙を以て濾し搾出液八三五立を得たり此汁液を低温に於て含利別状となるまで濃縮し其一般成分を檢したる成績次の如し

原汁液百分中 乾物百分中 全窒素を百として

水分 九三九七
固形物 六〇三〇
全窒素 〇〇五九七
蛋白質窒素 〇〇〇一一
糖のシッフム酸により沈澱する窒素 〇〇一〇一
其他の窒素 〇〇四八五
測定し得る窒素 〇〇二四〇
還元糖(葡萄糖として) 三九一

一〇〇〇〇
〇〇九九〇
〇〇一八
〇二六七五
〇八〇四五
〇三九八〇
六四八四三

一〇〇〇〇
一八四
一六九二
八一二四
四二〇一

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Figure 4. The first page from the article by Koga and Ohtake (1914) in the *Journal of the Tokyo Chemical Society*. To be read from right to left and from up to down

Thus a few years later, the Japanese scientist M. Wada, repeated their experiment in 1930 in the same laboratory (Agricultural Chemical Laboratory, Tokyo Imperial University). He isolated this new amino acid, defined its chemical formula and structure and proved that his observations concerning this new amino acid were correct by synthesizing citrulline. He named this amino acid "citrulline".^{15,29} He further demonstrated its isolation from the tryptic digestion of casein and possibly arginine.³⁰ He published his findings in a significant German journal of that period *Biochemische Zeitschrift*¹⁵ and in the *Proceedings of the Imperial Academy* in English.²⁹ Publishing in German and English was of great significance since it enhanced the swift acceptance of his results from fellow scientists.

The first isolation though by Koga and Ohtake²⁷ was acknowledged by other researchers over the following years. They attributed to Koga and Ohtake the first isolation from the juice of watermelon and denote that Wada performed the first sound study determining the chemical properties and structure of citrulline, as well as the name "citrulline".

First of all, Wada¹⁵ himself acknowledges their contribution to the first isolation of citrulline by saying "Sixteen years ago in this laboratory, Y. Koga and S. Odake³¹ isolated from the juice of watermelon a nitrogenous compound as colorless prisms, different from arginine and Glycocoll betaine ... The analysis yielded the empirical formula $C_6H_{13}N_3O_3$. Since then, the structure has not been elucidated".

Ackermann,³² who has been referenced elsewhere as the co-discoverer of citrulline with Wada,²⁵ mentioned in reference to citrulline, that, "such a substance had already been isolated from the watermelon, *Citrullus vulgaris*, by Y. Koga and S. Odake, and its structure had been fully described by Wada".

A few years later, Vickery³³ mentions "In 1914, Koga and Odake described the isolation of a substance $C_6H_{13}N_3O_3$ from the juice of watermelon. Aside from the fact that it formed a copper salt, little else was recorded. In 1930, Wada prepared the substance again and showed that its properties were best explained on the assumption that it is L-carbamido ornithine". Vickery³⁴ repeats his views once more several years later in an article on the discovery of amino acids.

Krebs and Henseleit³⁵ demonstrated that citrulline is an intermediate in the mechanism whereby urea is formed in the liver, signifying the importance of this amino acid in nitrogen metabolism,³⁶ citing first the work by Koga and Ohtake,²⁷ and then that of Wada.¹⁵

Fearon notes that "the carbamido-acid citrulline, isolated by Koga & Odake in 1914 from the watermelon, attracted no general attention until Wada (1930) established its constitution as α -amino- δ -carbamidovaleric acid".³⁶

Finally, Impellizzeri et al.,³⁷ while claiming that citrulline has been detected in a variety of plant sources and presumably is universal in plants (because of its role in arginine biosynthesis), cite the work by Koga and Ohtake²⁷ as the first to have isolated citrulline from the juice of watermelon.

Conclusion

In the present article, we demonstrated that before 1930, when modern citrulline, was first believed to be isolated, there was use of the term citrulline, signifying though a resin produced by *C. Colocynthis*. This citrulline is different from modern citrulline. However, neither was modern citrulline isolated in 1930 but somewhat earlier. Reviewing the original manuscripts, Koga and Ohtake²⁷ did indeed isolate citrulline for the first time and at least half a dozen other researchers cite their work. Even though their work didn't lead

to the determination of the structure and nature of citrulline, theirs was the first to isolate it. Our results have a certain historical and clinical significance since they lead to a hypothesis: Could this old citrulline-resin be considered by physicians as an alternative clinical useful purgative, when given subcutaneously or rectally?

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Conflict of interest statement: No competing interest

References

- 1) J. Nightingale and J. M. Woodward, 'Guidelines for management of patients with a short bowel', *Gut*, 55 (2006), iv1–iv12.
- 2) Jeremy Nightingale (ed.), *Intestinal Failure* (London, 2001).
- 3) Pascal Crenn, 'Citrulline et métabolisme protéique', *Nutrition clinique et métabolisme*, 22 (2008), 75–79; Pascal Crenn, Bernard Messing and Luc Cynober, 'Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction', *Clinical Nutrition*, 27 (2008), 328–339.
- 4) D. Rabier and P. Kamoun, 'Metabolism of citrulline in man', *Amino Acids*, 9 (1995), 299–316.
- 5) E. Curis, I. Nicolis, C. Moinard, S. Osowska, N. Zerrouk, S. Bénazeth and L. Cynober, 'Almost all about citrulline in mammals', *Amino Acids*, 29 (2005), 177–205.
- 6) Fang-nan Liu, Li Tan, Nan Luo, Feng Jiang, Yuan-xin Li and You-sheng Li, 'Determination of serum citrulline levels in normal Chinese people with HPLC', *Parenteral & Enteral Nutrition*, 11 (2004), 116–117.
- 7) Konstantinos C. Fragkos and Alastair Forbes, 'Are Citrulline Plasma Levels a Marker of Small Bowel Length and Absorptive Capacity? A Meta-Analysis', *UCL Biomedical Sciences Conference, 28/2-1/3 2011*, (2011).
- 8) C. Moinard, I. Nicolis, N. Neveux, S. Darquy, S. Bénazeth and L. Cynober, 'Dose-ranging effects of citrulline administration on plasma amino acids and hormonal patterns in healthy subjects: the Citrudose pharmacokinetic study', *British Journal of Nutrition*, 99 (2008), 855–862.
- 9) 'Progress of Medicine', *North Carolina Medical Journal*, 11 (1883), 38–49.
- 10) Acute Abdominal Pain
- 11) 'Glycerin and Citrullin Suppositories', *Medical Age*, 12 (1894), 115.
- 12) 'Queries and Minor Notes', *Journal of the American Medical Association*, 53 (1909), 310.
- 13) Leonard J. Hoening and James D. Boyle, 'The life and death of Ismar Boas', *Journal of clinical Gastroenterology*, 10 (1988), 16–24.
- 14) Ismar Boas, *Diseases of the intestines* (New York and London, 1904).
- 15) Mitsunori Wada, 'Über Citrullin, eine neue Aminosäure im Preßsaft der Wassermelone, *Citrullus vulgaris* schrad', *Biochemische Zeitschrift*, 224 (1930), 420–429.
- 16) A. Hiller, 'Ueber die subcutane Anwendung von Abführmitteln', *Zeitschrift für Klinische Medicin*, 4 (1882), 481–497.
- 17) P. Kohlstock, 'Ueber subcutane und rectale Anwendung von Abführmitteln', *Charité-Annalen*, 17 (1892), 283–294.
- 18) M. Chaturvedi, P. C. Mali and A. S. Ansari, 'Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* Schrad fruit in male rats.', *Pharmacology*, 68 (2003), 38–48.
- 19) Frederick Belding Power and Charles Watson Moore, 'The constituents of colocynth', *Journal of the Chemical Society, Transactions*, 97(Pt 1) (1910), 99–110.

- 20) H. El Khadem and M. M. A. Abdel Rahman, 'Constituents of the Fruit of *Citrullus colocynthis*', *Journal of the Chemical Society*, (Pt 4) (1963), 4991–4993; Natiq A. R. Hatam, Donald A. Whiting and Nahia J. Yousif, 'Cucurbitacin glycosides from *Citrullus colocynthis*', *Phytochemistry*, 28 (1989), 1268–1271; Natiq A. R. Hatam, Donald A. Whiting and Nahia J. Yousif, 'Lipids and Sterols of *Citrullus colocynthis*', *International Journal of Crude Drug Research*, 28 (1990), 183–184.
- 21) Jansen, 'Ueber Coloquinthen-Vergiftung', *Therapeutische Monatshefte*, 3 (1889), 39–41; D. Goldfain, A. Lavergne, A. Galian, L. Chauveinc and F. Prudhomme, 'Peculiar acute toxic colitis after ingestion of colocynth: a clinicopathological study of three cases', *Gut*, 30 (1989), 1412–1418.
- 22) Samuel Wyllis Bandler, *Medical Gynecology* (Philadelphia, 1915).
- 23) *Merck's 1907 Index* (New York, 1907), 143.
- 24) H. G. Windmueller and A. E. Spaeth, 'Source and fate of circulating citrulline', *American Journal of Physiology. Endocrinology and Metabolism*, 241 (1981), 473–480.
- 25) Hans Kornberg, 'Krebs and his trinity of cycles', *Nature Reviews Molecular Cell Biology*, 1 (2000), 225–228.
- 26) H. Mandel, N. Levy, S. Izkovitch and S. H. Korman, 'Elevated plasma citrulline and arginine due to consumption of *Citrullus vulgaris* (watermelon)', *Journal of Inherited Metabolic Disease*, 28 (2005), 467–472; Christophe Moinard and Luc Cynober, 'Citrulline: A new player in the control of nitrogen homeostasis', *Journal of Nutrition*, 137 (2007), 1621S–1625S.
- 27) Yotaro Koga and Ryo Ohtake, '[Study report on the constituents of squeezed watermelon]', *Tokyo Kagaku Kaishi [Journal of the Tokyo Chemical Society]*, 35 (1914), 519–528.
- 28) J. Stanlaw, The history of Japanese English contact *Japanese English: Language And The Culture Contact* (Aberdeen, Hong Kong, 2004), 45–82.
- 29) Mitsunori Wada, 'On the Occurrence of a New Amino Acid in Watermelon', *Proceedings of the Imperial Academy*, 6 (1930), 15–17.
- 30) Mitsunori Wada, 'Isolierung des Citrullins (δ -Carbamido-ornithin) aus tryptischen Verdauungsprodukten des Caseins', *Biochemische Zeitschrift*, 257 (1933), 1–7.
- 31) Wada (op. cit. ref. 29) possibly spells Ryo Ohtake's surname as Odake. This spelling is used by all the other authors citing their work after 1930. We have kept the original spelling, according to our translation, but Ohtake and Odake refer to the same individual.
- 32) D. Ackermann, 'Über den biologischen Abbau des Arginins zu Citrullin', *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, 203 (1931), 66–69.
- 33) H. B. Vickery, 'Evidence from Organic Chemistry Regarding the Composition of Protein Molecules', *Annals of the New York Academy of Sciences*, 41 (1941), 87–120.
- 34) H. B. Vickery, 'The History of the Discovery of the Amino Acids II. A Review of Amino Acids Described since 1931 as Components of Native Proteins', *Advances in Protein Chemistry*, 26 (1972), 81–171.
- 35) H. A. Krebs and K. Henseleit, 'Untersuchungen über die Harnstoffbildung im Tierkörper', *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, 210 (1932), 33–66.
- 36) William Robert Fearon, 'The carbamido diacetyl reaction: a test for citrulline', *Biochemical Journal*, 33(Pt 6) (1939), 902–907.
- 37) Giuseppe Impellizzeri, Sebastiano Mangiafico, Giovanna Oriente, Mario Piattelli, Sebastiano Sciuto, Ernesto Fattorusso, Silvana Magno, Ciro Santacroce and Donato Sica, 'Amino acids and low-molecular-weight carbohydrates of some marine red algae', *Phytochemistry*, 14 (1975), 1549–1557.

Appendix A

A bibliographic list of the sources mentioning the term “citrulline” from 1882 to 1930, before the discovery of the modern amino acid citrulline

No.	Source
<i>Dutch Texts</i>	
1	Nederlands tijdschrift voor geneeskunde. Houten: Bohn Stafleu van Loghum; 1894. vol. 38, p. 1010
2	Ritsema IC, Sack J, Greshoff M. Index phytochemicus. Amsterdam: Uitgave van het Laboratorium; 1905. p. 12
<i>English Texts</i>	
3	Morse WH. New therapeutical agents. Detroit: Davis; 1882. p. 183
4	The Cincinnati Lancet-Clinic. Cincinnati: J. C. Culbertson; 1882. vol. 48, p. 136
5	The London Medical Record. London: Hart; 1882. vol. 10, p. 490
6	American Medical Digest. New York; 1882. p. 156
7	The Louisville Medical News. Louisville: Ky; 1882. pp. 117–8
8	Medical Record. New York: W. Wood; 1882. vol. 22, p. 604
9	Chicago Medical Review. Chicago: Chandler & Engelhard; 1882. vol. 5–6, p. 563
10	The Medical Press & Circular. London; 1882. p. 268
11	The North Carolina Medical Journal. Charlotte, N. C.; 1883. vol. 11, p. 45
12	The Medical Times and Register. Philadelphia: Medical Publishing Company; 1883. vol. 13, p. 71
13	Pharmaceutical Journal and Transactions. London: Churchill; 1883. p. 343
14	Therapeutic Gazette. G. S. Davis; 1883. vol. 7, p. 380
15	Lescher FH. Recent materia medica: notes on their origin and therapeutics. London: Churchill; 1884. p. 27
16	Bruce JM. Materia medica and therapeutics, 1st ed. London: Cassell; 1884. p. 297
17	Medical and Surgical Reporter. Philadelphia; 1889. vol. 60, p. 498
18	Wood's Medical and Surgical Monographs. New York: W. Wood; 1889. vol. 3, p. 504
19	Meyer Druggist. St. Louis; 1891. p. 358
20	Braithwaite JO. Yearbook of Pharmacy. London: J.&A. Churchill; 1893. p. 220
21	American Veterinary Review. USA; 1893. vol. 17, p. 138
22	The Medical Age. Detroit: E. G. Swift; 1893. vol. 11, p. 322
23	Therapeutic Gazette. G. S. Davis; 1893. vol. 17, p. 704
24	Bulletin of Pharmacy. E. G. Swift; 1893. vol. 7, pp. 181, 279, 286
25	American Journal of Pharmacy. Philadelphia: Philadelphia College of Pharmacy; 1893. vol. 65, p. 225
26	The Medical Bulletin. Philadelphia: F. A. Davis; 1893. vol. 15, p. 310
27	Proceedings of the American Pharmaceutical Association at the Annual Meeting. Scio, Ohio; 1893. vol. 41, p. 450
28	Western Druggist. Chicago; 1893. vol. 15, p. 150
29	Chemist & Druggist. London: Morgan Brothers; 1893. vol. 42, p. 380
30	Yearbook of Pharmacy. London: J.&A. Churchill; 1893. p. 220
31	Pacific Medical Journal. San Francisco; 1893. vol. 36, p. 562
32	The Cincinnati Lancet and Clinic. Cincinnati: Culbertson; 1893. vol. 70, p. 540
33	The Western Medical Reporter. Chicago; 1893. vol. 15, issue 1, p. 278
34	The Journal of the British Homoeopathic Society. London; 1893. vol. 1, p. 101
35	Proceedings of the ... annual meeting of the Kansas Pharmaceutical Association. Marysville: Hoadley & Hackman; 1893. vols. 14–20, p. 119
36	Stevens AA. A Manual of Therapeutics. Philadelphia: Saunders; 1894. p. 126
37	The Medical Age. Detroit: E. G. Swift; 1894. vol. 12, pp. 31, 115, 532
38	Annual of the Universal Medical Sciences. Philadelphia: F. A. Davis; 1894. vol. 5, p. A76
39	The Year-book of Treatment. Lea Brothers; 1894. vol. 10, p. 124
40	The Veterinary Magazine. Philadelphia; 1894. vol. 1, p. 223
41	Shoemaker JV. A practical treatise on materia medica and therapeutics: with especial reference to the clinical application of drugs, 3rd ed. Philadelphia: F. A. Davis; 1895. p. 359
42	Boas I. Diseases of the intestines (Trans. S Basch). New York: Appleton; 1901. p. 253
43	Therapeutic Monthly. Philadelphia: Medical Journal Union; 1901. vols. 1–2, p. 88
44	The Pharmaceutical Era. New York: D. O. Haynes; 1902. vol. 27, p. 15

No.	Source
45	Fantus B. A text book on prescription writing and pharmacy. Chicago: Chicago Medical Book Co.; 1905. p. 251
46	Gould GM. A dictionary of new medical terms. Blakiston; 1905. p. 185
47	Wilcox RW. Materia medica and pharmacy. Philadelphia: Blakiston; 1905. pp. 543–4
48	Wilcox RW. Pharmacology and therapeutics, 6th ed. Philadelphia: Blakiston; 1905. p. 706
49	de Médicis Sajous CE. Sajous's analytical cyclopædia of practical medicine, 3rd ed. Philadelphia: F. A. Davis; 1906. vol. 2, p. 275
50	Culbreth DMR. A Manual of materia medica and pharmacology, 4th ed. Philadelphia: Lea Brothers; 1906. p. 602
51	Wood HC. Therapeutics: its principles and practice, 13th ed. Philadelphia: Lippincott; 1906. p. 651
52	Croftan AC. Clinical Therapeutics. Chicago: Cleveland Press; 1907. p. 456
53	Pharmaceutical Era. The Era Dose Book. Philadelphia: Haynes; 1907. p. 7
54	Merck's 1907 Index. Merck & Co.; 1907. p. 143
55	MacEwan P. The art of dispensing: a treatise on the methods and processes involved in compounding medical prescriptions, 8th ed. London: Chemist and Druggist; 1908. p. 421
56	Coblentz V. The newer remedies, including their synonyms, sources, tests, solubilities, incompatibles, medicinal properties and doses as far as known, together with such proprietaries as have similar titles, 4th ed. Apothecary Publishing Co.; 1908. p. 36
57	The Cincinnati Lancet and Clinic. Cincinnati: Culbertson; 1908. vol. 100, pp. 136, 366
58	Billings F. Diseases of the digestive system. New York and London: Appleton; 1910. p. 729
59	Therapeutic Gazette. Detroit: G. S. Davis; 1910. vol. 34, p. 556
60	The Encyclopaedia Britannica: a dictionary of arts, sciences, literature and general information. Encyclopaedia Britannica; 1910. vol. 6, p. 696
61	Beal JH. Elementary Principles of the Theory and Practice of Pharmacy: Animal and vegetable drugs. Scio, Ohio: J.H. Beal; 1911. vol. 3, p. 149
62	Bandler SW. Medical gynecology, 3rd ed. Philadelphia and London: Saunders; 1914. p. 470
63	Veterinary Materia Medica and Therapeutics, 8th ed. Chicago: American Veterinary Publishing Co.; 1919. p. 416
64	Prinz H. Dental materia medica and therapeutics: with special reference to the rational application of remedial measures to dental diseases: a textbook for students and practitioners, 5th ed. St. Louis: C. V. Mosby; 1920. p. 612
65	Aaron CD. Diseases of the digestive organs, with special reference to their diagnosis and treatment, 3rd ed. Philadelphia: Lea & Febiger; 1921. p. 227
66	Gardner W. Chemical synonyms and trade names: a dictionary and commercial handbook, 3rd ed. C. Lockwood and Son; 1926. p. 89
<i>French Texts</i>	
67	L'Union Pharmaceutic. Paris; 1882. vol. 23, p. 563
68	Revue des Sciences Médicales en France et a l'étranger. Paris: Masson; 1883. vol. 21, pp. 517–8
69	Répertoire de Pharmacie. Paris; Baillière; 1883. vol. 11, p. 422
70	Journal de Médecine, de Chirurgie et de Pharmacologie. Bruxelles: Lamertain; 1883. vol. 77, p. 598
71	Bulletin Général de Thérapeutique. Paris: Bureau du Journal; 1884. p. 430
72	Journal de Médecine, de Chirurgie et de Pharmacologie. Bruxelles: Lamertain; 1884. vol. 79, p. 174
73	Bourneville, Bricon. Manuel des injections sous-cutanées. Paris: Progrès Medical; 1885. p. 187
74	Bouchut E. Compendium Annuaire de Therapeutique. Paris; 1885. p. 69
75	Dujardin-Beaumetz G, Egasse E. Les plantes médicinales indigènes et exotiques. Paris: O. Doin; 1889. p. 201
76	Bouley H-M, Sanson A, Reynal J. Nouveau dictionnaire pratique de médecine, de chirurgie, et d'hygiène vétérinaires. Paris: Labé; 1890. p. 366
77	Revue Médicale, Louvain: Charles Peeters; 1892. vol. 11, p. 516
78	L'Union Pharmaceutic. Paris; 1892. vol. 33, p. 504
79	Hayem G. Leçons de thérapeutique Paris: Masson; 1893. vol. 4, p. 531
80	Gazette de Gynécologie. Paris: Odoin; 1893. vol. 8, p. 159
81	Bulletin général de thérapeutique médicale, chirurgicale, obstétricale et pharmaceutique. Paris: G. Doin & cie; 1893. vols. 124–125, p. 568
82	de Maurans G. Compendium moderne de médecine pratique. Paris: Maloine; 1894. p. 414
83	Recueil de Médecine Vétérinaire. Paris: Vigot Éditions; 1894, vol. 71, pp. 71–3
84	Journal d'Hygiène. Paris; 1894. vol. 20, p. 96
85	Haller A. L'Industrie Chimique. Paris: Baillière; 1895. p. 204
86	Revue de Médecine Vétérinaire. Toulouse (France). Écoles nationales vétérinaires de Lyon et de Toulouse; 1895. vol. 52, p. 707
87	Planchon G, Collin E. Les drogues simples d'origine végétale. Paris: O. Doin; 1896. p. 300
88	Cagny P. Formulaire des vétérinaires praticiens. Paris: Baillière; 1897. p. 107

No.	Source
89	Stokvis BJ. Leçons de pharmacothérapie. Haarlem, Bohn & Paris: Octave Doin; 1898. vol. 2, pp. 267, 274–5
90	Le Moniteur Scientifique. Paris: G. Quesneville; 1900. vol. 56, p. 722
91	Manquat A. Traité élémentaire de thérapeutique de matière médicale et de pharmacologie. Paris: J.-B. Bailliere et fils; 1903. vol. 1, p. 732
92	Cohin E. Traité de toxicologie végétale. Paris: O. Doin; 1907. p. 76
<i>German Texts</i>	
93	Zeitschrift für Klinische Medizin. Berlin: A. Hirschwald; 1882. vol. 4, pp. 481–97
94	Kongress für Innere Medizin. Verhandlungen. Wiesbaden; 1882. vols. 1–2, p. 217
95	Zentralblatt (Centralblatt) für Klinische Medizin. Leipzig; 1882. vol. 3, p. 159; vol. 14, pp. 455–6
96	Medizinische Neuigkeiten fuer Praktische Aerzte. Erlangen: Palm & Enken; 1882. vol. 32, issue 28, pp. 222–3
97	Centralblatt für die Medicinischen Wissenschaften. Berlin; 1882. vol. 20, p. 603
98	Archiv der Pharmazie. Berlin: Deutschen Apotheker-Vereins; 1882. vol. 220, issue 9, p. 688
99	Deutsche Medizinal-Zeitung. Berlin: Grosse; 1882. vol. 3, p. 310
100	Harnack E. Lehrbuch der Arzneimittellehre und Arzneiverordnungslehre. Hamburg: Voss; 1883. p. 356
101	Karsten H. Illustriertes Repetitorium der Pharmaceutisch-Medicinischen Botanik und Pharmacognosie. Berlin: Springer; 1883. p. 214
102	Jahresbericht über die Leistungen und Fortschritte in der Gesammten Medicin. Berlin: A. Hirschwald; 1883. p. 317
103	Jahrbuch für Practische Aerzte. Berlin: A. Hirschwald; 1883. vol. 6, p. 143
104	Nothnagel H, Rossbach MJ. Handbuch der Arzneimittellehre, 5th ed. Berlin: A. Hirschwald; 1884. pp. 572, 582–3
105	Boehm R. Lehrbuch der allgemeinen und speciellen Arzneiverordnungslehre für studierende, Aerzte und Apotheker. Jena: Fischer; 1884. p. 502
106	Medizinisch-Chirurgische Rundschau. Wien: Urban und Schwarzenberg; 1884. vol. 23, p. 593
107	Jahresbericht über die Fortschritte der Pharmakognosie, Pharmacie und Toxikologie. Göttingen: Vandenhoeck und Ruprecht; 1884. vols. 16–17, p. 948
108	Jahresbericht der Pharmazie. Göttingen: Vandenhoeck & Ruprecht; 1886. vols. 18–19, p. 948
109	Ewald CA. Handbuch der allgemeinen und speciellen Arzneiverordnungslehre. Berlin, Hirschwald; 1887. p. 753
110	Koch A. Encyclopädie der gesammten Thierheilkunde und Thierzucht. Wien: Perles; 1888. vol. 5, p. 79
111	Liebreich MEO. Compendium der Arzneiverordnung: nach dem Arzneibuch für das deutsche Reich und den neuesten fremden Pharmacopoeen. Berlin: Fischer; 1891. p. 703
112	Geissler E, Moeller J. Real-encyclopädie der gesammten pharmacie. Wien und Leipzig: Urban & Schwarzenberg; 1891. vol. 10, p. 916
113	Charité-Annalen. Berlin: Hirschwald; 1892. vol. 17, pp. 283–94
114	Pharmazeutische Zentralhalle für Deutschland. Berlin: Dresden Steinkopff; 1892. vol. 33, p. 689
115	Zeitschrift für therapie: mit einbeziehung der electro- und hydrotherapie. Wien: Weiss; 1892. vol. 10, issue 22, pp. 173–4
116	Leipziger populäre Zeitschrift für Homöopathie. Leipzig: W. Schwabe; 1892. vol. 23, p. 222
117	Wissenschaftliche Drogenkunde. Berlin: Gaertner; 1892. vol. 2, p. 425
118	Cloetta A, Filehne W. Dr. A. Cloetta's Lehrbuch der Arzneimittellehre und Arzneiverordnungslehre, 8th ed. Leipzig: Mohr; 1893. p. 232
119	Schmidt's Jahrbücher der in- und ausländischen gesammten Medizin. Leipzig: Wigand; 1893. vol. 238, issue 1, p. 17
120	Excerpta Medica. Leipzig: Sallman; 1893. vol. 2, p. 141
121	Therapeutische Monatshefte, Berlin: Springer; 1893. vol. 7, p. 34
122	Pharmaceutische Rundschau. New York: Pharmaceutical Review Pub. Co.; 1893. vol. 11, p. 20
123	Archiv für wissenschaftliche und practische Thierheilkunde. Berlin: A. Hirschwald; 1893. vol. 19, p. 427
124	New Yorker Medicinische Monatsschrift. New York: Medical Monthly Pub. Co.; 1893. vol. 5, p. 278
125	Jahrbuch der praktischen Medizin: Kritischer Jahresbericht für die Fortbildung der praktischen Ärzte. Stuttgart: F. Enke; 1893. p. 289
126	Zentralblatt für die Gesamte Therapie. Wien: M. Perles; 1893. vol. 11, p. 16
127	Archiv für wissenschaftliche und practische Thierheilkunde. Berlin: A. Hirschwald; 1894. vol. 20, pp. 10, 11, 21
128	Jahresbericht über die Leistungen auf dem Gebiete der Veterinär-Medicin. Berlin: A. Hirschwald; 1894. vols. 13–14, pp. 151, 179, 181
129	Jahrbuch der praktischen Medizin: Kritischer Jahresbericht für die Fortbildung der praktischen Ärzte. Stuttgart: F. Enke; 1894. p. 772
130	Karsten H. Flora von Deutschland, Oesterreich und der Schweiz: Gera-Untermhaus: Köhler; 1895. vol. 2, p. 459
131	Deutsche Zeitschrift für Tiermedizin und vergleichende Pathologie. Leipzig: Vogel; 1895. vols. 21–22, p. 356
132	Penzoldt F. Handbuch der speciellen Therapie der innerer Krankheiten in sechs Baenden. Jena: G. Fischer; 1896. vol. 4, p. 526

No.	Source
133	Real-Encyclopadie der gesammten Heilkunde. Berlin: Urban & Schwarzenberg; 1896. vol. 11, p. 604
134	Thoms HFM. Die Arzneimittel der organischen Chemie: für Ärzte, Apotheker, und Chemiker. Berlin: Springer; 1897. p. 53
135	Eulenburg A. Lehrbuch der allgemeinen Therapie und der therapeutischen Methodik. Wien: Urban & Schwarzenberg; 1898. vol. 1, p. 166
136	Liebreich MEO. Encyklopaedie der Therapie. Berlin: A. Hirschwald; 1898. vol. 2, p. 809
137	Nevinný J. Allgemeine und specielle Arzneiverordnungslehre für Studierende und Aerzte. Leipzig: Deuticke; 1900. p. 182
138	Ebstein W, Schwalbe J, Braun. Handbuch der praktischen Medicin. Stuttgart: F. Enke; 1900. vol. 2, p. 616
139	Andreae JM. Zusammenstellung neuer Arzneimittel: mit kurzen Bemerkungen über Herkommen, Zusammensetzung, Wirkung und Dosirung, 3rd ed. Leipzig: W. Engelmann; 1901. p. 39
140	Kionka H. Grundriss der Toxicologie. Leipzig: Veit; 1901. p. 110
141	Die Therapie der Gegenwart: Medizinisch-chirurgische Rundschau für praktische Ärzte. Wien: Urban & Schwarzenberg; 1901. p. 311
142	Zeitschrift des allgemeinen Oesterreichischen Apotheker-Vereines. Wien: Im Selbstverlage des Vereines; 1901. vol. 39, p. 26
143	Ewald CA. Klinik der Verdauungskrankheiten. Berlin: Hirschwald; 1902. vol. 3, p. 121
144	Mindes J. Manuale der neuen Arzneimittel: für Apotheker, Ärzte und Drogisten, 4th ed. Zürich: Art Institut Orell Füssli; 1902. p. 66
145	Pharmazeutische Zentralhalle für Deutschland. Berlin: Dresden Steinkopff; 1902. vol. 43, p. 318
146	E. Merck (Firm). Preis-Liste der chemischen Fabrik von E. Merck in Darmstadt: Vorzugspreise; 1903. p. 81
147	Arends G. Neue arzneimittel und pharmazeutische spezialitäten, einschliesslich der neuen drogen, organ-und serum-preparate. Berlin: Springer; 1903. p. 114
148	Pamphlets on Biology: Kofoid collection. 1903. vol. 409, pp. 33, 80
149	von Lengerken OFF. Arzneibuch für Mediziner: Handbuch zur Beurteilung und zur selbständigen Aufstellung von Rezepten, im Anschluss an das Arzneibuch für das Deutsche Reich, 4. Ausg. Leipzig: Veit; 1904. p. 204
150	Fortschritte der praktischen und wissenschaftlichen Pharmazie. Berlin: Urban & Schwarzenberg; 1905. p. 87
151	Schnirer MT. Taschenbuch der Therapie mit besonderer Berücksichtigung der Therapie an den Berliner, Wiener u.a. deutschen Kliniken, 2nd ed. Wien; 1906. p. 187
152	Rodari P. Grundriss der medikamentösen Therapie der Magen- und Darmkrankheiten. Wiesbaden: Bergmann; 1906. p. 152
153	Robert R. Lehrbuch der intoxicationen. Stuttgart: F. Enke; 1906. vol. 2(Pt. 2), p. 561
154	Leyden E, Llemperer F. Deutsche Klinik am Eingange des zwanzigsten Jahrhunderts. Berlin: Urban & Schwarzenberg; 1907. p. 499
155	von Lengerken OFF. Handbuch neuerer Arzneimittel. Frankfurt: Alt; 1907. p. 199
156	Zweig W. Die Therapie der Magen- und Darmkrankheiten. Berlin & Wien: Urban & Schwarzenberg; 1907. p. 288
157	Penzoldt F. Lehrbuch der klinischen Arzneibehandlung. Jena: G. Fischer; 1908. p. 561
158	Hager H. Hager's Handbuch der pharmaceutischen Praxis für Apotheker, Ärzte, Drogisten und Medicinalbeamte. Berlin: Springer; 1908. p. 231
159	Fröhner E. Lehrbuch der Arzneimittellehre für Tierärzte, 8th ed. Stuttgart: Enke; 1909. p. 440
160	Kahane M. Der Arzneitherapie der Gegenwart: Die neuesten Arzneimittel und ihre Anwendung in der ärztlichen Praxis. Berlin: Urban & Schwarzenberg; 1910. p. 224
161	Wegele C. Die Therapie der Magen- und Darmerkrankungen. Jena: G. Fischer; 1911. p. 141
162	Roth O. Die Arzneimittel der heutigen Medizin: mit therapeutischen Notizen zusammengestellt für praktische Ärzte und Studierende der Medizin, 11th ed. Würzburg: Kabitzzsch; 1911. p. 137.
163	Zörnig H. Arzneidrogen als nachschlagebuch für den gebrauch der apotheker, ärzte, veterinärärzte, drogisten und studierenden der pharmazie. Leipzig: W. Klinkhardt; 1913. vol. 1, p. 205
164	Muspratt JS. Encyklopädisches Handbuch der technischen Chemie. F. Vieweg & Sohn; 1915. vol. 3(Pt. 1), p. 92
165	Heinigke C. Handbuch der homöopathischen Arzneiwirkungslehre. Leipzig: Schwabe; 1922. p. 204
166	Heffter A, Heubner W. Handbuch der experimentellen Pharmakologie. Berlin: Springer; 1924. vol. 2 (Pt. 2), p. 1654
167	Skandinavisches Archiv für Physiologie. Berlin: Walter De Gruyter & Co.; 1926. vols. 49–50, p. 158
168	Winterstein EH, Trier G. Die Alkaloide: eine Monographie der natürlichen Basen. Berlin: Borntraeger; 1927. p. 814
<i>Italian Text</i>	
169	Balsamo-Crivelli R. Boccacino: racconto, 2nd ed. Bari: Laterza; 1921. p. 27
<i>Swedish Text</i>	
170	Rosendahl HV. Lärobok i farmakognosif. Upsala: Schultz; 1897. p. 400

Appendix B

Translation of the article of Koga and Ohtake (1914) from Japanese into English.

Koga Y, Ohtake R. Study report on the constituents of squeezed watermelon.

Journal of the Tokyo Chemical Society [Tokyo Kagaku Kaishi] 1914; **35**: 519–28.

古賀彌太郎, 大嶽 了. 西瓜搾汁の成分研究報告. 東京化学會誌 1914; **35**: 519–528.

Study report on the constituents of squeezed watermelon

by Bachelors of Agriculture

Yotaro Koga

Ryo Ohtake

Red-coloured interiors of 23 ripe watermelon (*Citrullus vulgaris* Schrad) (weight 180 kg) were separated and pressed to get juice from them. After filtering through paper, 83.55 litres of liquid were left. The liquid was concentrated at a low temperature until it had a sherbet-like condition. The general results of its analysis are as follows:

	Original juice	Dried provision	All nitrogens
	100%	100%	100%
water	93.97000		
Solid (substance)	6.03000	100.0000	
all nitrogens	0.05970	0.9900	100.00
protein nitrogen	0.00110	0.0180	1.84
nitrogen which is precipitated by phosphorus wolfram acid	0.01010	0.1675	16.92
other nitrogens	0.04850	0.8045	81.24
nitrogen which can be measured by Formol method	0.02400	0.3980	42.01
reducing sugar (as grape sugar)	3.91000	64.8430	
inverted sugar	4.54000	75.2900	
cane sugar	0.59900	9.9340	
pentose	0.06416	1.0640	
ash content	0.18370	3.0460	
acidity original juice 100 millilitre equivalent to 0.0844g caustic baryta			
as malic acid	0.06600	1.0940	

Quantitative table of ash content

	original juice (100%)	dried provision (100%)	
ash content	0.1837	3.4660	100.000
K ₂ O	0.1222	2.0270	66.522
Na ₂ O	0.0030	0.0497	1.634
CaO	0.0081	0.1340	4.355
MgO	0.0071	0.1180	3.865
Fe ₂ O ₃	0.0003	0.0049	0.164
P ₂ O ₅	0.0108	0.1790	5.879
SO ₃ (sulfuric acid)	0.0074	0.1230	4.028
SiO ₂ (silicic acid)	0.0032	0.0530	1.742

Experimentation

1. Separation of acids

A spoonful of the mentioned sherbet-like substance was diluted to make a litre of liquid. In the liquid, basic acetic acid lead solution was added until precipitation was yielded. After being separated from the liquid and cleaned with water, the precipitation was distributed thoroughly in the proper amount of water and decomposed through hydrogen sulphide. Lead sulphide precipitation yielded from the decomposition was removed and then free hydrogen sulphide was eradicated at low temperature.

The same operation was repeated again to get colourless free acid liquid and then the liquid was highly concentrated and left in a cool place for some days. As no crystal was yielded from the concentrated liquid, a part of it was diluted with water, added with copper hydroxide, boiled and filtered. The filtered solution then formed big pillar shaped crystals (0.9 g) through concentration. This substance was not carbonized by heat. By removing copper by hydrogen sulphide and adding nitric acid and molybdenum acid liquid to free acid solution, it had the reaction which was peculiar to phosphoric acid. Therefore, it was proved that they were crystal forms of copper phosphate.

After confirming that new crystal form was no longer separated out from the mother liquid from which copper phosphate crystal forms had already been removed, copper was precipitated by hydrogen sulphide and removed. All the acid liquids were then brought together and concentrated. When thick ammonia solution was added to them, white pillar-shaped ammonium phosphate (0.5 g) was formed and filtered. Sometimes the filtered solution was then added with ammonia water. While being stirred, the solution was concentrated to sherbet-like condition. After being added with alcohol and left as it was, it formed pillar shaped crystals (0.8 g) and melted at 178 degrees Celsius (no correction). It was dried at 100 degrees Celsius in a vacuum and analyzed as follows:

0.1565 g substance	0.1839 g carbon dioxide	0.0836 g water		
0.1178 g substance	9.5 ml nitrogen (23 degree Celsius, 760 mm)			
	carbon	hydrogen	nitrogen	
calculated figure (C ₄ H ₉ NO ₅)	31.79	5.96	9.27	
experimental figure	32.04	6.13	9.04	

Namely the analysis showed the same figure that acid ammonium salt of malic acid had. As the crystals' mother liquid had quite a small amount, more specific experiment was not done.

2. Separation of nitrogen containing compounds

After excessive lead was removed by passing hydrogen sulphide through the filtrate which had been yielded by the mentioned basic lead acetate, the filtrate was concentrated in low pressure and temperature. Then its' hydrogen sulphide was driven out from the filtrate and amount of the filtrate was about 700 ml.

While sodium carbonate was added to the filtrate for neutralization, mercury acetate solution was added to it by slow degree until limit of precipitation formation in it. Then alcohol was added to it to sink all the precipitation in it and the filtrate was filtrated with suction and the precipitation was washed well with alcohol. Then the precipitation was distributed in water and resolved by hydrogen sulphide. For resolving the precipitation of mercury in it, hydrogen sulphide was used a few times until complete decomposition. After that all the filtrate was collected and vaporized. Hydrogen sulphide was driven away from the filtrate, and its' amount was made to be about 500 ml. Sulphuric acid was added to it to have 5% density solution and also phosphorus wolfram acid was added to make precipitation in it.

After being filtrated with suction and washed well, the precipitation was decomposed with baryta by an ordinary method. Excessive baryta was precisely removed with sulphuric from the filtrate. Then the filtrate was concentrated, added with picric acid and cooled down with ice to make a lot of crystals (3.6 g yielded amount). When they were recrystallized from warm water, they had beautiful needle-like shapes at 204 melting point (no correction). They were dried and analyzed as follows.

0.0994 g substance	0.1299 g carbon dioxide	0.0442 g water		
0.1813 g substance	37.6 ml nitrogen (14 degree Celsius, 748 mm)			
0.1567 g substance	0.0889 g picric acid			
	carbon	hydrogen	nitrogen	picric acid
calculated figure (C ₆ H ₁₄ N ₄ O ₂ C ₆ H ₃ N ₃ O ₇)	35.73	4.21	24.32	56.82
experimental figure	35.64	4.94	23.91	56.73

Namely, it matched with arginine picrate.

It was found that when the mother liquid of arginine picrate was concentrated in low temperature, a lot of crystals were precipitated. 24 hours later the crystals were collected and washed well with alcohol ether, but this substance did not combine with picric acid and was precipitated in a free state. Therefore, the substance was dissolved further in water and picric acid was removed by shaking the solution with ether.

With boneblack the solution was decoloured and alcohol (in the same container) was added and left as it was. Then, by low degrees, white pillar-shaped large crystals were yielded (yielded amount 0.8 g). In 168 degree Celsius (no correction), it was decomposed with bubbles. In a vacuum (100 degree Celsius) it was dried and analyzed as follows.

I	{	0.1804 g substance	30.8 ml nitrogen (19 degree Celsius, 760 mm)	
		0.0960 g substance	0.1274 g carbon dioxide	0.0635 g water
II	{	0.1100 g substance	21.16 ml nitrogen (28 degree Celsius, 752 mm)	
		0.1094 g substance	0.1440 g carbon dioxide	0.0677 g water

		carbon	hydrogen	nitrogen
calculated figure (as $C_4H_{10}N_2O_3$)		35.84	7.46	20.89
experimental figure	{ I	36.19	7.35	19.55
	II	35.88	7.04	20.89

This substance had the mentioned experimental figure. Its' water solution had neutrality and no property of an organic base. When it burned, no ash content was left. When it was boiled with ninhydrin solution, it had the blue-coloured reaction as an amino acid did. When it had red heat in a narrow tube and a piece of hydrochloric acid soaked-wood was closed near, the substance turned red like pirol. And when the substance was boiled with copper hydroxide, copper salt was yielded. At 232 degree Celsius, the substance turned to red with bubbles and decomposed itself. When it was heated with 10% hydrochloric acid, it separated ammonia. Other properties were not examined further due to material meagre.

To the mentioned substance's mother liquid, sulphuric acid was added to make it become acid. To remove picric acid from the liquid, it was shaken several times with ether. And sulphuric acid was precisely removed by baryta from it. When the liquid was concentrated and boiled with copper hydroxide, it yielded copper salt which hardly melted in water. When the copper was collected and recrystallized from boiling water, large panel-shaped crystals were yielded. They turned to red colour at 257 degree Celsius, producing bubbles. They were dried and analyzed in a vacuum (100 degree Celsius) as follows.

I	{	0.0764 g substance	13.2 ml nitrogen (19 degree Celsius, 764 mm)	
		0.0889 g substance	0.1125 g carbon dioxide	0.0543 g water
		0.1357 g substance	0.0267 g copper	
II	{	0.0713 g substance	0.0895 g carbon dioxide	0.0438 g water
		0.1026 g substance	18 ml nitrogen (21 degree Celsius, 751 mm)	

		carbon	hydrogen	nitrogen	copper
calculated figure (as $C_{12}H_{28}N_6O_6Cu$)		34.64	6.34	20.21	15.30
experimental figure	{ I	34.51	6.78	19.88	15.70
	II	34.23	6.82	19.53	

Then this copper salt was decomposed and made to become an extricated substance. The substance was analyzed as follows.

0.1024 g substance	0.1510 g carbon dioxide	0.0680 g water		
0.1209 g substance	24.75 ml nitrogen (25 degree Celsius, 756 mm)			
	carbon	hydrogen	nitrogen	
calculated figure (as $C_6H_{15}N_6O_7$)	40.68	8.47	23.72	
experimental figure	40.21	7.37	22.56	

This substance had a sweet taste and its' properties were very much like the mentioned substance. A part of the nitrogen was able to be titrated by the Formol method. The copper salt was melted in water more difficultly than the mentioned one. Water solution of the extricated substance showed reddish purple colour by copper sulphate solution and caustic soda solution. It precipitated copper salt for a day or two. The precipitation of phosphorus wolfram acid had beautiful pillar-shaped crystals and melted easily by an excess of the phosphorus wolfram acid. It was also found that the precipitation had much relation with sulphuric acid's concentration then.

By the above reason, the authors added baryta to the filtrate of the precipitation of phosphorus wolfram acid to remove the phosphorus wolfram acid and sulphuric acid. After removing precisely excessive baryta by sulphuric acid, they concentrated it and continued to add sulphuric acid to it until it had 7% density. When phosphorus wolfram acid was added gradually to it, it was found that in addition to crystalline precipitation, oily substance was yielded. Therefore quickly these 2 kinds of precipitations were separately filtrated with suction and the crystalline precipitation was decomposed by baryta by an ordinary method. After the baryta in it was removed by sulphuric acid, the solution was concentrated. Totally the same crystals as the mentioned substance was yielded (total yielded amount 0.7 g).

As the oily precipitation became solidified when it was left for some time, it was crystallized after being separated from filter paper, decomposed by baryta, concentrated and added with alcohol. White needle-shaped crystals (amount 0.5 g) were yielded. They were decomposed at 180 degree Celsius while bubbles were produced. They were the copper salt which had salty taste and melted in water easier than the copper salt of the mentioned substance. It turned red with bubbles at 248 degree Celsius. The copper salt was dried and analyzed in a vacuum (100 degree Celsius) as follows.

0.1510 g substance	0.1896 g carbon dioxide	0.0755 g water		
0.1083 g substance	18.3 ml nitrogen (20 degree Celsius, 769 mm)			
	carbon	hydrogen	nitrogen	
calculated figure (as $C_{12}H_{28}N_6O_6Cu$)	34.64	6.34	20.21	
experimental figure	34.24	5.55	19.44	

And the analysis of the extricated substance showed as follows.

0.1420 g substance	27.3 ml nitrogen (17 degree Celsius, 766 mm)
0.1056 g substance	20.7 ml nitrogen (20 degree Celsius, 766 mm)

	nitrogen
calculated figure (as $C_6H_{15}N_3O_7$)	23.72
experimental figure	22.42, 22.50

Namely the analysis showed that it matched with the mentioned substance. But as the other properties did not match, it could be determined that both substances were isomers.

As the properties of the mentioned 3 substances were very much like a polypeptide, the authors planned to hydrolyze and form ester. But the materials for it were trifling and satisfied results could not be achieved. The authors hope that in the future, further study on it will be done. Phosphorus wolfram acid and sulphuric acid were removed from the filtrate of the phosphorus wolfram acid precipitation by baryta and excessive baryta was removed precisely by sulphuric acid from the filtrate. The filtrate was concentrated and a small amount of sherbet-like substance was produced from it. Then water was added to it for having alkalinity in it. Drops of dimethyl sulphate were added for methylation. Then when picric acid was added, glycol betaine picric acid salt (0.05 g) was gotten at 180 degree Celsius melting point. Its' yielded amount was trifling and analysis on it was not done.

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