Reactions with substituted malonic acids are still in progress.

Esters of Succinic Acid.—Twenty-five g. of dimethyl succinate, boiling at 196.9°, was treated with 0.4 g. of potassium in 250 g. of ethyl alcohol. 21.6 g. of diethyl ester, boiling chiefly at 216.4 to 216.6°, was obtained, a yield of 74%.

From 25.48 g. of diethyl succinate, 12.8 g. of dimethyl ester boiling at 196° to 200° was obtained, a yield of 61%.

Esters of Phthalic Acid.—Neither dimethyl nor diethyl phthalate entered into reaction to an appreciable extent under the conditions used. This is in harmony with the results obtained with other *ortho* substituted esters.

Esters of Terephthalic Acid.—Ten g. of dimethyl terephthalate, 0.4 g. of potassium, and 130 g. of ethyl alcohol were used. 9.1 g. of diethyl terephthalate was obtained, a yield of 83%.

10 g. of diethyl terephthalate subjected to similar treatment, gave 7.5 g. of dimethyl ester, an 86.2% yield.

Summary.

As a result of these experiments the conclusion may be drawn that the direct replacement of one alkoxyl group by another may be carried out at room temperature, with a very small amount of alkaline reagent. This is of importance for ester preparation in general, but especially for that of unstable esters which cannot be prepared at high temperatures, or under the influence of acid reagents.

Under the conditions here described, the reaction is restricted to primary alcohols. The change from methyl esters to those of higher primary alcohols, and of ethyl esters to methyl, can be brought about in good yields with esters of all the acids studied with the exception of those which usually show "hindrance" to ester formation.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE IDAHO STATE EXPERIMENT STATION.]

THE APPLICATION OF THE VAN SLYKE METHOD TO HYDROLYZED PROTEIN EXTRACTS OF SILAGE CROPS.[1]

BY RAY E. NEIDIG AND ROBT. S. SNYDER.

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Newer knowledge of the rôle that amino acids play in nutrition has led invesitgators to attempt to analyze the protein molecule of feeding stuffs to learn of its composition, especially the percentage of amino acids. Since the introduction of Van Slyke's[2] method for determining the amino acids in pure proteins, investigators have studied the composition of feeding stuffs by means of applying the Van Slyke method directly to a hydrolyzed extract of the original material. Chief among

[1] Published with the permission of the Director of the Idaho State Experiment Station.

[2] D. D. Van Slyke, *ibid.*, 10, 15 (1911–12).

these investigators are Grindley, Eckstein and Slater.[1,2,3,4] These investigators used several concentrates such as wheat, barley, oats, white soy beans, cotton seed meal, tankage, blood meal and alfalfa hay. They found, in all cases a considerable amount of nitrogen in the humin fraction, resulting from the hydrolysis of the protein in acid solution and realized this fact to be a source of error. However, they concluded that "the Van Slyke method for the determination of the chemical group characteristics of the amino acids of proteins can be applied directly to the quantitative determinations of feeding stuffs with at least a fair degree of accuracy." The application of the Van Slyke method to such heterogeneous material led to numerous criticisms by other investigators as to the possibility of applying this method to material other than pure proteins, for which it was originally intended. Much literature has been published both for and against applying the Van Slyke[2] method to heterogeneous materials. Gortner[5,6,7,8] and his associates have carried out an investigation carefully planned to show the effect of various substances of the carbohydrate and aldehyde groups, on the formation of humin and on the other nitrogen fractions when protein material is hydrolyzed with acid. These studies have enlightened to a great degree our knowledge of the formation of humin. All such investigations are of great value and one should not be easily discouraged when studying the complex protein structure, neither should he expect to reach that last stage in protein knowledge too hastily.

Soon after the publication of Grindley and Slater,[9] the senior author in 1916 began a study of some forage crops commonly used for silage. It was desired to study the crops before and after undergoing the fermentation in the silo. In applying the Van Slyke method to these silage crops it was realized that the results might be greatly influenced by other materials present, such materials being entirely different from pure proteins. It was, however, desired to apply the Van Slyke method to hydrolyzed protein extracts of these substances for the purpose of comparison only, the writers fully realizing that all such percentages of fractions would represent the nitrogen found in these fractions and need not necessarily be, as in the case of the bases, pure lysine, arginine, or histidine,

[1] H. S. Grindley, W. E. Joseph and M. E. Slater, THIS JOURNAL, **37**, 1778 (1915).
[2] H. S. Grindley and M. E. Slater, *ibid.*, **37**, 2762 (1915).
[3] H. S. Grindley and H. C. Eckstein, *ibid.*, **38**, 1425 (1916).
[4] H. C. Eckstein and H. S. Grindley, *J. Biol. Chem.*, **37**, 373 (1919).
[5] R. A. Gortner and M. J. Blish, THIS JOURNAL, **37**, 1630 (1915).
[6] R. A. Gortner, *J. Biol. Chem.*, **26**, 177 (1916).
[7] R. A. Gortner and G. E. Holm, THIS JOURNAL, **39**, 2477 (1917).
[8] R. A. Gortner and G. E. Holm, *ibid.*, **39**, 2737 (1917).
[9] *Loc. cit.*

but rather the nitrogen found in these fractions when analyzed by the Van Slyke method.

The original methods described by Grindley and Slater[1] were used in the analyses of the two silages, alfalfa and peas.

The results were obtained in triplicate and appear below.

TABLE I.

	Ammonia N. %	N. humin. %	Total N of bases. %	Arginine N. %	N. cystine. %	N histidine. %	N. lysine. %	Amino N in filtrate. of bases. %	Non-amino N in filtrate from bases. %	Total N. %
Pea silage.....	20.24	10.45	19.20	7.74	1.43	1.90	8.13	43.97	4.67	98.11
Pea silage.....	20.31	10.63	18.71	7.36	1.44	1.74	8.17	44.26	5.08	99.11
Pea silage.....	20.51	10.33	18.72	7.41	1.40	1.68	8.23	44.56	4.60	99.22
Average.....	20.35	10.47	18.86	7.50	1.42	1.77	8.17	44.2	4.78	98.93
Alfalfa silage..	12.91	15.77	21.65	2.82	1.99	9.06	7.78	41.22	8.43	99.98
Alfalfa silage..	12.90	14.22	21.60	2.82	1.99	9.06	7.73	41.22	7.58	97.52
Alfalfa silage..	13.57	14.05	22.06	2.95	1.95	9.16	8.10	41.10	7.39	98.27
Average silage.	13.12	14.68	21.79	2.86	1.97	9.09	7.87	41.08	7.80	98.92
*Average of alfalfa hay.....	8.44	15.79	20.10	7.68	0.88	7.44	4.10	44.02	9.79	98.14

* Analyzed by Grindley and Slater.[1]

It is not our purpose to discuss these results at this time. They are given with a view of calling attention to the fact that in an analysis of alfalfa silage a very considerable amount of humin nitrogen occurs in both cases. Further discussion will be undertaken later in this paper.

No further work was carried on in the analysis of silage crops for some time, due to war conditions and also because it was realized that the method needed revision in order that the large percentage of humin nitrogen be lowered.

In 1919, Eckstein and Grindley[1] modified their former method to a large extent. The essential features of the revized method are as follows: (1) extraction of the feeding stuffs with ether and then with cold absolute alcohol; (2) the conversion as far as possible of the insoluble carbohydrates into soluble carbohydrates by boiling the feeding stuffs with 0.1% hydrochloric acid; (3) the separation of the greater part of the soluble proteins before hydrolysis from the soluble carbohydrates by neutralizing the mineral acid and precipitating with alcohol; (4) the hydrolysis of the small portion of the proteins remaining in solution with the soluble sugars by boiling with 5% instead of 20% hydrochloric acid for only a short time.

By means of this new procedure, Eckstein and Grindley lowered the quantity of humin nitrogen considerably. They obtained the following percentages of humin nitrogen by the new method: corn 3.2%; wheat 3.4%; oats 4.5%, and barley 3.9%. The results on these same substances when analyzed by the old method were as follows: corn 9.8%; wheat 9.2%; oats 9.9%, and barley 8.8% humin nitrogen.

[1] *Loc. cit.*

TABLE II.

The Nitrogen of Amino Acids of Feeding Stuffs.

Results Expressed in Milligrams of Nitrogen.

Before siloing.	After siloing.	Grams of anhydrous feeding stuffs hydrolyzed.	Ammonia nitrogen.	Melanin N. (a b c fraction.)	Lime fraction.	Amino N of bases.	Non-amino N of bases.	Total N of bases.	Cystine N.	Arginine N.	Histidine N.	Lysine N.	Amino N in filtrate from bases.	Non-amino N in filtrate from bases.	Total N in filtrate from bases.	Ether N.	Alcohol N.	Actual T. N.	T. N.[a] found by addition of fractions.
Peas		25	66.97	56.32	31.80	65.82	75.59	141.42	13.94	64.51	40.95	22.01	250.39	50.99	301.40	3.64	7.56	601.31	609.12
	Peas	25	105.72	26.90	27.32	71.68	66.55	141.10	15.66	40.52	54.40	30.53	239.68	37.68	277.38	10.79	67.67	679.77	656.87
Clover		25	70.75	61.22	25.64	57.72	69.84	128.13	13.08	63.54	33.82	18.08	236.20	41.18	277.38	..	...	572.58	563.12
	Clover	25	61.36	58.14	28.44	50.80	65.78	116.58	6.64	49.00	43.64	17.30	229.15	33.65	262.80	9.25	21.50	582.05	558.08
Wheat		50	49.60	60.94	32.36	37.00	59.59	96.59	9.74	48.18	35.32	3.35	175.60	57.73	233.33	..	7.14	471.57	479.96
	Wheat	50	58.00	57.44	25.22	41.12	45.38	86.50	9.00	34.74	23.04	8.28	173.67	80.46	254.14	4.76	24.80	493.86	510.86
Alfalfa		25	63.19	60.66	23.12	54.27	87.48	141.74	10.34	61.94	61.68	6.35	256.17	44.8	300.98	5.04	8.41	626.67	603.13
	Alfalfa	25	48.19	48.47	28.30	67.26	55.75	123.01	8.38	41.14	37.48	36.01	232.74	10.59	243.34	3.64	62.20	571.40	557.16
Oats		50	43.29	50.44	21.72	35.60	50.90	86.50	11.02	41.14	23.75	10.58	152.32	22.17	174.49	..	6.30	404.18	382.73
	Oats	50	54.78	50.86	29.56	36.52	46.94	83.46	9.90	32.84	26.02	14.67	132.60	26.68	159.28	8.13	47.91	444.54	433.97
	Corn	50	85.60	74.25	43.71	45.40	72.65	115.19	12.66	46.58	56.70	2.10	218.24	57.12	275.36	7.99	23.40	649.22	625.50
	Wheat and vetch	37.5	74.95	95.55	38.81	62.40	113.76	176.16	7.94	85.94	74.10	8.18	269.32	90.11	359.43	..	11.21	784.84	756.10
	Wheat and vetch	37.5	48.19	47.63	28.30	32.89	49.61	82.49	7.97	23.54	48.07	2.94	130.67	33.81	164.48	4.48	35.44	418.76	411.03
	Wheat and peas	37.5	90.92	59.12	46.65	84.62	124.04	208.66	10.17	111.54	60.71	26.24	336.53	43.71	380.23	5.04	18.63	860.36	809.28
	Oats and peas	37.5	61.64	53.66	33.76	83.82	71.20	155.03	16.60	73.14	24.65	40.63	264.02	46.15	310.18	..	8.55	632.40	622.84
Sunflower		50	50.44	46.09	39.65	39.02	48.75	87.78	9.90	46.90	20.53	10.44	212.17	52.78	264.95	..	9.39	526.78	498.30
	Sunflower	50	113.48[b]	50.26	43.57	37.65	61.00	98.66	11.44	52.36	32.70	2.15	262.18	132.32	394.50	10.37	16.88	880.80	852.79

[a] Actual total nitrogen represents the actual amount present in crops. Total nitrogen found by addition of fractions is the summing up of all fractions found during the analysis.

[b] Phosphotungstic melanin nitrogen = 25.078 mg. = 2.84%.

TABLE III.—THE NITROGEN OF AMINO ACIDS OF FEEDING STUFFS.

Results Expressed in Per cent. of Nitrogen.

Before siloing.	After siloing.	Ammonia nitrogen.	Melanin N. (a b c fraction.)	Melanin N. (Lime fraction.)	Amino N of bases.	Non-amino N of bases.	Total N of bases.	Cystine N.	Arginine N.	Histidine N.	Lysine N.	Amino N in filtrate from bases.	Non-amino N in filtrate from bases.	Total N in filtrate from bases.	Ether N.	Alcohol N.	T. N. found by analysis.
Peas		11.14	9.36	5.29	10.95	12.56	23.52	2.32	10.72	6.81	3.66	41.65	8.48	50.13	0.61	1.26	101.2
	Peas	15.55	3.96	4.02	10.55	9.78	20.75	2.31	5.96	8.00	4.49	35.23	5.54	40.8	1.59	9.96	96.7
Clover		12.34	10.69	4.48	10.06	12.19	22.37	2.28	11.09	5.91	3.16	41.24	7.18	48.40	0	0	98.3
	Clover	10.54	9.97	4.88	8.73	11.29	20.02	1.14	8.41	7.5	2.97	39.38	5.78	45.10	1.59	3.69	96.0
Wheat		10.52	12.92	6.86	7.85	12.63	20.50	2.06	10.22	7.49	0.71	37.26	12.24	49.50	0	1.52	101.6
	Wheat	11.74	11.64	5.12	8.33	9.19	17.52	1.82	7.04	4.67	1.68	35.20	16.30	51.56	0.97	5.02	103.4
Alfalfa		10.08	9.68	3.69	8.70	13.95	22.60	1.65	9.88	9.85	1.01	40.90	7.15	48.01	0.81	1.35	96.2
	Alfalfa	8.43	8.50	4.96	11.77	9.76	21.52	1.47	7.20	6.56	6.31	40.72	1.86	42.60	0.63	1.09	97.6
Oats		10.70	12.47	5.37	8.8	12.58	21.36	2.72	10.15	5.87	2.62	37.62	5.48	43.10	0	1.56	94.6
	Oats	12.31	11.34	6.66	8.22	10.55	18.75	2.02	7.38	5.85	3.30	29.81	5.99	35.8	1.83	10.76	97.6
	Corn	13.18	11.44	6.74	6.99	11.19	17.74	1.95	7.16	8.74	0.33	33.62	8.8	42.41	1.23	3.60	96.4
	Wheat and vetch	9.55	12.20	4.95	7.95	14.50	22.43	1.01	10.95	9.45	1.03	34.32	11.49	45.80	0	1.44	96.45
	Wheat and vetch	11.5	11.38	6.76	7.84	11.85	19.66	1.9	5.62	11.48	0.70	31.20	8.07	39.23	1.07	8.46	98.1
	Wheat and peas	10.56	6.87	5.43	9.84	14.43	24.23	1.18	12.96	7.06	3.05	39.11	5.08	44.42	0.59	2.17	94.0
	Oats and peas	9.75	9.48	5.33	13.25	11.25	24.5	2.62	11.55	3.89	6.43	41.71	7.29	49.00	0	1.35	98.5
Sunflower		9.58	8.75	7.56	7.41	9.26	16.56	1.88	8.91	3.91	1.98	40.30	10.02	50.22	0	1.78	94.8
	Sunflower	12.88	17.05	4.94	4.28	6.93	11.20	1.30	5.94	3.71	0.24	29.79	15.01	44.39	1.18	1.92	96.9

Since such a large reduction in the humin nitrogen had been secured by Eckstein and Grindley when their new method was used, it was thought that possibly a reduction in humin nitrogen could be obtained by applying the new method to the silage crops. It was realized, however, that these silage crops differed from the cereal grains, analyzed by Eckstein and Grindley, in that they contained a much greater percentage of cellulose.

With a view of securing more information on the protein of forage crops before and after siloing, the crops named in Table II were analyzed by the new method.

It will be noted that Tables II and III contain figures on the percentages of nitrogen in the ether and alcohol extracts. Nitrogen was determined in these fractions because it was suspected that some nitrogen compounds were extracted by the anhydrous ether and alcohol. Nitrogen apparently was not determined on these fractions by Eckstein and Grindley. In our work, this is the only modification of the new method as proposed by Eckstein and Grindley. It is possible that no appreciable amount of nitrogen was extracted in their work since they used the cereal grains and concentrated feeding stuffs. An inspection of Tables II and III corroborates this assumption for by far the greater amount of nitrogen was usually found in the silage rather than in the same crop that had not undergone the silage fermentation.

It is interesting to note that in some of the legume silages a large amount of nitrogen was found, especially in the alcohol extract of silage made from peas and from alfalfa. In the case of legumes mixed with grains the amount of nitrogen found in the ether and alcohol extract is much less; in silage made from the grains alone, such as oats and wheat, there is usually more nitrogen found in the ether and alcohol extracts than in those silages made from mixtures of legumes and grains. The reason for this fact may be due to a slight decomposition of protein material in peas, alfalfa, oats and wheat when siloed individually. It is commonly known that a legume such as alfalfa does not always silo successfully when siloed alone. This is due to the absence of sufficient fermentable carbohydrates necessary to form the required acidity, resulting in a partial protein decomposition. In the case of the grains, oats and wheat, when siloed individually there may be a slight decomposition of protein caused by a short period of unfavorable bacterial action, resulting from a small amount of oxygen contained in air held in the hollow stems which can not be entirely removed from the silo even by carefully packing. The fact that the crops themselves, before siloing, do not show any appreciable amount of nitrogen in the ether and alcohol extract is a further indication that some slight change has taken place in the protein during silage formation.

To determine what percentage of nitrogen in the alcohol and ether extracts was ammonia, the extracts from the following silages, wheat, alfalfa, oats, wheat and vetch were separately treated with distilled water, the solutions made slightly acid with sulfuric acid, and the ether and alcohol evaporated. After their removal the solutions were made alkaline with calcium hydroxide, aerated, and the ammonia collected in standard acid. The data follow.

TABLE IV.

	Ether extract. % ammonia.	Alcohol extract. % ammonia.
Wheat silage	100.0	23.4
Oat silage	34.5	32.1
Alfalfa silage	61.6	14.2
Wheat and vetch silage	28.1	16.3

The table shows that a considerable amount of nitrogen in the extracts is in compounds that yield ammonia when aerated from an alkaline solution.

Table V is given for the sake of comparing the results secured when the original and the new method, suggested by Eckstein and Grindley, is applied to pea and alfalfa silage, and the results of the new method when applied to alfalfa hay. As a check, the results on alfalfa hay obtained by Grindley using the old method, are included. In this last instance the sources of alfalfa hay are entirely different, while in the case of the pea and alfalfa silage the samples are identical. The results follow:

TABLE V.—COMPARISON OF THE OLD AND NEW METHODS.

	N as NH_3.	N humin.	Total N of bases.	Arginine.	Cystine.	Histidine.	Lysine.	Amino N in filtrate.	Non-amino N in filtrate from bases.	Total N.
Pea Silage										
Old method....	20.35	10.47	18.86	7.50	1.42	1.77	8.17	44.2	4.78	98.9
New method...	16.10	8.25	21.48	6.17	2.38	8.27	4.65	36.45	5.73	96.8
Alfalfa Silage										
Old method....	13.12	14.68	21.79	2.86	1.97	9.09	7.87	41.08	7.80	98.9
New method...	8.43	13.46	21.52	7.20	1.47	6.56	6.31	40.72	1.86	97.6
Alfalfa Hay										
Old method.... (Analyzed by Grindley)	8.44	15.79	20.1	7.68	0.88	7.47	4.10	44.02	9.79	98.14
New method... (Analyzed by Neidig and Snyder)	10.08	13.37	22.6	9.88	1.65	7.85	1.01	40.9	7.15	96.2

A comparison of the humin nitrogen obtained when portions of feeding stuffs are analyzed by the old and new method, shows that the new method usually gives a slightly lower percentage of humin nitrogen. The lowering of the humin nitrogen, however, is very slight. The cause of the high humin nitrogen even when the new method is used is no doubt due to to the high percentage of cellulose. Gortner[1] has shown that protein

[1] *Loc. cit.*

material when hydrolyzed in the presence of cellulose results in the production of a considerable quantity of humin nitrogen. Even when the new method, suggested by Eckstein and Grindley, is applied to forage crops, the results are not greatly changed for, although the soluble sugars and the hydrolyzed starch may be removed, there still remains such an abundance of cellulose that a large amount of humin results during hydrolysis.

A critical study of Tables II and III shows that the figures representing the total nitrogen of the bases are slightly less in crops after siloing. When a comparison of the individual bases is made the results show some glaring irregularities. Not only does there seem to be no relation between the amount of individual bases in the forage crops before and after siloing, but the percentages of the bases are variable.

In the case of lysine, the amount formed may be more or less in silage than in the original crop. In alfalfa, for instance, the lysine shows an increase from 1.01 to 6.31%. It is of course unreasonable to suppose that this is actually the case.

Since it is generally known that there are nitrogen compounds carried over into the bases in an analysis of heterogeneous materials that affect the true determination of the individual amino acids and since lysine and histidine are determined by calculation rather than direct analysis, it is reasonable to suppose that the greatest error will be found in the histidine and lysine fractions.

In all the results on the above heterogeneous materials, the reader must bear in mind that although the nitrogen found in each fraction reacts similarly to bases found in pure protein, it does not necessarily follow that it may be justly called pure lysine or histidine nitrogen, but rather it is the nitrogen found in the separate fractions after the conditions of the hydrolysis have been carried out.

In almost all cases the percentage of total nitrogen is usually below 100%. One source of loss was noted too late in the analytical work to secure the actual percentage lost. In the case of the phosphotungstic acid precipitate of the bases, a dark colored substance usually formed with the bases. This substance has been observed by Gortner and is called phosphotungstic humin. It was found that the greater part of this nitrogen is recovered in the barium phosphotungstic precipitate. Analysis of this precipitate in the case of sunflower silage showed 2.84% of humin. Had the precipitates from all the forage crops been saved and analyzed the percentage of phosphotungstic humin would no doubt have been sufficient to raise materially the total nitrogen recovered toward 100%. The results, it appears, do not warrant the hope that the protein of forage crops, containing such a large quantity of cellulose which it is impossible to remove, can be successfully analyzed by the

Van Slyke method. It is believed, however, that in such concentrates as contain but little cellulose, a fair interpretation of the protein molecule can be secured when analyzed by the new method of Eckstein and Grindley. These results are submitted with a view of indicating what the Van Slyke method shows when applied directly to forage crops high in cellulose.

MOSCOW, IDAHO.

NEW BOOKS.

Zur Feier der Entdeckung der Röntgenstrahlen vor fünfundzwanzig Jahren (The Twenty-fifth Anniversary of the Discovery of Roentgen Rays), Die Naturwissenschaften, No. 50, Vol. 8. Julius Springer, Berlin, 1920. 30 pp., 1 plate, 17 figures. 19.5 × 27 cm. Price, M. 3.

The whole number of Die Naturwissenschaften for the 10th of December, 1920, is devoted to a symposium commemorating the 25th anniversary of the discovery of Roentgen rays. The separate articles have been written by many of the foremost German scientists who have contributed to progress in this field. They are as follows.

The discovery of Roentgen rays twenty-five years ago; by W. Wien.

Roentgen rays and therapy; by Max-Leve Dorn.

The history of the development of Roentgen tubes; by Paul Knipping.

In what way are we justified in speaking of a microscopic delineation of minute structure by means of Roentgen rays? by M. v. Lane.

Roentgen rays and crystallography; by Friedrich Rinne.

The fundamentals of Roentgen ray spectroscopy; by Ernest Wagner.

On the significance of Roentgen rays in the study of atomic structure; by W. Kossel.

The gain to chemistry from the physical study of Roentgen rays; by Paul Pfeiffer.

It is really astonishing to consider how much this capital discovery of Roentgen has meant to our intellectual development. It, along with the discovery of radium, has revolutionized our whole concept of matter, and has opened vast regions to still more daring flights of the human intellect.

ARTHUR B. LAMB.

Eminent Chemists of Our Time. BENJAMIN HARROW, Ph.D., Associate in Physiological Chemistry, Columbia University, New York. D. Van Nostrand Company, 8 Warren Street, New York, 1920. xvi + 248 pp. 13.5 × 21.5 cm. Price, $2.50 net.

There are eleven of these charming scientific biographies in this attractive volume. It has been truly said by some distinguished writer, whose name I cannot now recall, that the only true history is biography. Prof. Harrow's book lends much support to this theory. The history of chemistry told in tales of chemists is not only attractive, but it is alive; there is no dead wood in it. The present volume is indeed a part of a large volume in which it was intended to put a description of the work as well as the life of the chemists selected for this purpose. The work part is promised for another volume.