steam-bath. Weigh the beaker with contents and from the weight thus obtained calculate the percentage of oil in the seed.

Make the combined acid extracts of the alkaloids distinctly alkaline with 15% sodium hydroxide solution and extract with chloroform, 3 portions of 15 cc. each, or until the aqueous layer gives a negative test for alkaloids. (The test with Wagner's reagent is made in slightly acid solution.) Make the combined chloroform extracts up to exactly 100 cc. and pour over the previously obtained marc in a stoppered flask. After several minutes add 5 cc. of *N* sodium hydroxide solution and shake the flask at frequent intervals for two hours. Filter the contents in a 4-inch Büchner funnel (using a rapid paper), catching the liquid in a suitable vessel such as a calibrated graduate. To avoid evaporation cover the funnel with a watch glass, and do not use suction. Collect as large an aliquot as possible, which will usually be 75 cc.

Extract the chloroform solution repeatedly with 5% sulphuric acid until the last extract fails to give a positive test for alkaloids. Six extractions of 15 cc. each are usually sufficient. Make the aqueous solution distinctly alkaline with sodium hydroxide and extract with chloroform, 3 portions of 15 cc. each, or until the alkaloids are completely removed. For further purification, which is usually necessary and is indicated by the yellow color in the chloroform solution, extract the latter with 5% sulphuric acid as before, again make it alkaline and extract with chloroform. Wash the chloroform solution once with 5–10 cc. of slightly alkaline water and filter through paper into a weighed beaker, washing the separator and funnel well with the solvent. Evaporate the chloroform by means of an air current and moderate heat, add a few cubic centimeters of alcohol, and again evaporate. Warm the beaker a few minutes in the oven (100° C.), cool in a desiccator and weigh. From the weight of the residue calculate the percentage of alkaloids in the seed.

A sample of *D. consolida* and one of *D. staphisagria* were analyzed by this method, with the following results:

D. consolida.		*D. staphisagria.*	
Oil, per cent.	Alkaloids, per cent.	Oil, per cent.	Alkaloids, per cent.
28.3	1.06	34.9	1.30
28.7	1.01	34.7	1.35
28.6	....	35.2	1.22
28.7	....	35.4	1.27

CHEMICAL EXAMINATION OF "PYRIDIUM" AND "MALLOPHENE."

(BRANDS OF PHENYLAZO-ALPHA-ALPHA-DIAMINOPYRIDINE HYDROCHLORIDE.)

BY GEORGE W. COLLINS, PH.C., SC.D.*

Pyridium and Mallophene are proprietary names for two genito-urinary antiseptics recently introduced to the medical profession. The former is marketed by Merck & Co., Inc., Rahway, N. J.; the latter by the Mallinckrodt Chemical Works, St. Louis, Mo. Both Pyridium and Mallophene belong to the azo group

* A contribution from the American Medical Association Chemical Laboratory.

of dyes. Azo dyes have been used in medicine for many years, including such well-known therapeutic agents as the "Scarlet R compounds."

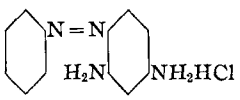
Pyridium, it is stated, was originated by Professor Iwan I. Ostromislensky,[1] who since then has come to America from Russia to give this country the benefit of his researches.[2]

Pyridium was presented to the Council on Pharmacy and Chemistry of the American Medical Association for inclusion in New and Nonofficial Remedies.[3] It was in this connection that the Chemical Laboratory was asked to investigate its chemical composition.

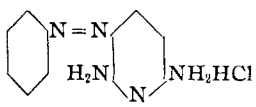
According to the presentation,[4] the product is: "phenylazo-alpha-alpha-diaminopyridine hydrochloride," $C_6H_5N:NC_5H_2N(NH_2)_2HCl$. It is prepared by[5] coupling benzenediazonium chloride with alpha-alpha-diaminopyridine in hydrochloric acid solution and subsequent crystallization from water.

Tchitchibabin and Zeide[6] in their work on compounds containing the pyridine nucleus apparently were the first to prepare the base of this azo dye. The base was described by them as being of an orange-red color and as appearing to have the formula $C_6H_5N:NC_5H_2N(NH_2)_2$, the positions of the amino (NH_2) groups being the same as in Chrysoidine, $C_6H_5N:NC_6H_4(NH_2)_2$.

A comparison of the structural formulas of diaminoazobenzene hydrochloride (Chrysoidine), which at one time was used in the treatment of trypanosomiasis and phenylazo-alpha-alpha-diaminopyridine hydrochloride (Pyridium), illustrates their similarity and conversely their chemical differences.



Chrysoidine



Pyridium

It will be noted that Pyridium differs from Chrysoidine in that it contains a pyridine nucleus instead of a benzene, hence the compound contains five nitrogen atoms in place of four.

SPECIMENS EXAMINED.

The specimens subjected to investigation were: *a.*—A specimen of Pyridium, presented by Merck & Co., Inc., in 1928, which was examined crystallographically;

[1] Iwan I. Ostromislensky, "The Scientific Basis of Chemotherapy," Part I.

[2] Recently Professor Ostromislensky has sponsored to the medical profession another new genito-urinary antiseptic, Serenium-Squibb, which is claimed to be ethoxy-diaminoazobenzene hydrochloride. [Q. & M. Notes, *J. A. M. A.*, 94 (May 31, 1930), 1783.]

[3] Generally abbreviated "N. N. R." A volume published annually by the Council on Pharmacy and Chemistry of the American Medical Association. It describes new remedies of merit which are not official in the United States Pharmacopœia.

[4] Pyridium was first presented about March 1, 1928, by Merck & Co., Inc., distributor, and it was stated that "Pyridium is composed of the monohydrochlorides of Beta- and Gamma-phenylazo-alpha-alpha-diaminopyridine, $C_6H_5N:NC_5H_2N(NH_2)_2HCl$." Some months later the Rare Chemicals, Inc., 100 Fifth Avenue, New York, N. Y., introduced Azamine to the dental trade, claiming it was a colloidal condensation product of the beta and gamma isomers of phenyl-azo-alpha-alpha-diaminopyridine hydrochlorides (Pyridium) especially adapted for dental use.

[5] Manufactured by the Pyridium Corporation of New York (Merck & Co., Inc., distributor), U. S. patents 1,680,108 and 1,680,109.

[6] *J. Russ. Phys.-Chem. Soc.*, 46 (1914), 1216–1236.

b.—Specimens of Pyridium[1] received from the distributor about two years later, on which this chemical examination was based; *c.*—Specimens of Mallophene[2] purchased on the open market. Mallophene was introduced while this investigation was under way. According to the label, it is manufactured by "Roosevelt Chemical Co., Inc." and distributed by the Mallinckrodt Chemical Works. Due to the similarity in the claims and in the origin of the product,[3] it was deemed advisable to include it in this study to determine whether or not it was identical with Pyridium.

CRYSTALLOGRAPHIC PROPERTIES.

Specimens of the products were investigated as to their crystalline character to ascertain, first, their status as definite chemical entities or mixtures and, second, the comparative values of the different brands. The collaboration of Dr. Albert J. Walcott, Professor of Mineralogy and Crystallography, Northwestern University, was obtained. Portions of the two different specimens of Pyridium and the one of Mallophene were subjected to crystallographic examination. Dr. Walcott reported as follows:

CRYSTALLOGRAPHIC PROPERTIES OF PYRIDIUM AND MALLOPHENE.

First Specimen (Report Made June 10, 1928).—Pyridium is a crystalline substance showing double refraction. The crystal fragments are very small and irregular in form. The slides examined did not show any well-formed crystals.

A very striking optical property is that of *pronounced pleochroism,* showing yellow, brownish red and opaque absorption characteristics.

Since there were no well-defined crystals or cleavage fragments, indices of refraction were determined only approximately:

One index of refraction was found to be higher than 1.740 and lower than 1.770.

One index was higher than 1.610 and lower than 1.640.

No limits were obtained for a third index because the absorption for that position was so strong that the crystal fragment was opaque.

Second Specimen (Report Made February 12, 1930).—This specimen is of a somewhat different character than the specimen of two years ago. The crystal fragments are slightly larger. They appear to be *"cleaner"* and more sharply defined. There is a marked difference in the per cent of an "impurity" or a second entity. In the specimen submitted two years ago there was present an appreciable amount of well-defined crystal fragments of a second entity. Such fragments are practically absent from the new sample. In ten slides, only two showed a couple fragments of this nature, while several fragments were always to be found in any slide made of the old sample. From my examination I would say that the second specimen of Pyridium is of purer quality than the first.

Mallophene.—The specimen of Mallophene is largely composed of a very fine crystalline powder, too fine to determine quantitatively any optical properties. This fine crystalline powder shows *double refraction* and also *pleochroism.* It is brownish red in color.

Mixed with this powder are well-defined crystal fragments, a second entity, of a substance which shows a very strong double refraction, is biaxial, optically positive, and has a small optical

[1] The labels of the containers of the product bore the same control numbers, hence they were pooled and used as specimen for analysis.

[2] Mallophene [Q. & M. Notes, *J. A. M. A.*, 93 (Dec. 28, 1929), 2044] was stated to be phenylazo-alpha-alpha-diaminopyridine hydrochloride. The product does not stand accepted by the Council on Pharmacy and Chemistry. It is *not* the policy of the Council on Pharmacy and Chemistry to recognize a second proprietary name for identically the same substance.

[3] The containers of Mallophene bore the same control numbers on the trade labels, hence they were pooled and used as a sample for analysis in this investigation.

axial angle. It shows a dispersion of optic axis, red > violet, and shows pleochroism. $\beta = 1.600 \pm 0.002$.

There is also present an appreciable per cent of irregular fragments which are isotropic in character.

CHEMICAL EXAMINATION.

The specimens of Merck & Co., Inc., were labeled Pyridium-Phenylazo-Alpha-Alpha-Diamino-Pyridine Hydrochlorides and those of the Mallinckrodt Chemical Works were designated as Mallophene[2]-Phenylazo-alpha-alpha-diamino-pyridine hydrochloride.

The color and appearance of the two products differed slightly: the Mallinckrodt specimen was a medium red, fine, microcrystalline powder; the Merck product was a dark red-violet but appeared to be a slightly coarser powder than Mallophene. Both specimens were odorless and possessed a slightly bitter taste. The solubilities of the products (1 Gm. in 250 cc. of water) differed somewhat; both were red in color. The solution of the Mallinckrodt product contained a very slight residue of floating particles (suggestive of filter fibre), while that of the Merck specimen was faintly turbid. The reaction of the aqueous solutions appeared to be neutral to litmus and congo paper but, as was expected, both products were distinctly acid when determined by the electrometric method. When subjected to thermal analysis, the Mallinckrodt specimen was found to melt at 233° to 234° C., with decomposition, while that of Merck & Co. melted at 237° to 238° C., also with decomposition, and a mixture of the two appeared to melt at 232° C., with decomposition.

SOME CHEMICAL REACTIONS.

On digesting the specimens with diluted hydrochloric acid, small, dark red crystals or scales separated in both instances; on decanting the supernatant liquid, followed by the addition of water to the crystalline precipitate, the compound formed dissolved. This very likely was due to the formation of an unstable di-hydrochloride; in these respects, the products of the two firms appeared to be similar.

On neutralizing aqueous solutions of the respective products with diluted ammonia, there resulted a brownish yellow, crystalline precipitate, which was subsequently recrystallized from water. The crystals obtained from the Mallinckrodt product melted at 136° to 137° C., while those from the Merck specimen melted at 138° to 139° C.; a mixture of the two melted at 136.5° to 137° C., which indicated that the crystalline substances were identical; these substances, according to the literature,[1] appear to be the free base, phenylazo-alpha-alpha-diaminopyridine, which melts at 137° to 138° C.

Upon reducing the compounds with zinc dust and diluted hydrochloric acid, aniline[2] was identified as one of the components of each of the products examined. This behavior is characteristic of diaminoazobenzene as well, the hydrochloride of which is known commercially as "Chrysoidine."

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[1] *Apoth.-Ztg.*, 44 (Nr. 17, 1929), 254.

[2] Tchitchibabin and Zeide, *J. Russ. Phys.-Chem. Soc.*, 46 (1914), 1216–1236, expected the base, phenylazo-α-α-diaminopyridine, upon reduction to yield aniline and triaminopyridine, α-α-β-$C_5H_2N(NH_2)_3$.

TABLE I.—LOSS ON DRYING.

Brand.	Method employed.[1]	Intervals of Drying and Weighing. 6 hrs.	24 hrs.	96 hrs.	216 hrs.	332 hrs.	572 hrs.
Mallophene	Sulphuric acid	0.41[2]	0.53	0.69		Constant	
	100° C.	1.08	1.42	1.57	1.64	1.76	1.96
Pyridium	Sulphuric acid	0.04	0.21			Constant	
	100° C.	0.11	0.18	0.26	0.29	0.45	0.65

[1] Specimens were exposed over sulphuric acid in a partially exhausted desiccator and in an electric oven at 100° C.

[2] Figures are reported in terms of percentages.

Moisture determinations carried on over a period of hours indicated that the two products differ a little. The specimens exposed over sulphuric acid in a desiccator with a partial vacuum obtained constant weight in a relatively short period of time, while those that were dried in an electric oven at 100° C., gradually lost weight. The Mallinckrodt specimen yielded the greater loss (see Table I).

The p_H curves of the electrometric titrations[1] were interesting. They were performed (*a*) on the undried substance, (*b*) on the specimens that had been exposed over sulphuric acid in a desiccator with a partial vacuum and (*c*) on specimens that had been previously dried in an electric oven at 100° C. for 572 hours. All curves of the titration with tenth-normal sodium hydroxide solution were found to be characteristic of an acidic substance, possessing only one sudden rise or break.[2] In the case of the undried specimens this break corresponded to an amount of hydrogen chloride less than the stoichiometrical amount for a dihydrochloride but slightly more than the amount necessary for a monohydrochloride. The curves on the dried material, whether it was dried in a partial vacuum over sulphuric acid or in an oven at 100° C., showed a sudden rise or break that corresponded stoichiometrically to that given by a monohydrochloride. It appears that the products were monohydrochlorides containing only slight amounts of uncombined hydrogen chloride. The Mallinckrodt product contained a small excess of hydrogen chloride.

The results of the quantitative determinations are recorded in Table II.

[1] The usual hydrogen electrode and calomel cell were used in these determinations.

[2] Our attention was called by Merck & Co., Inc., to an unpublished report on Pyridium prepared by a consulting chemist, who states that Pyridium would be reduced by hydrogen when a hydrogen electrode was placed in its solution and, therefore, that the electrometric titration "was scientifically precluded." The determinations made by me previous to this information show that the titration was not "scientifically precluded." Close checks were obtained on the specimens, which agreed well with theory, with the figures calculated from the chloride (Cl^-) determinations and with the colorimetric titrations (for comparative figures see Table II). The latter is not as "clean-cut" as the electrometric, due obviously to difficulty of a sharp color end-point.

The purpose of determining the hydrogen chloride after drying was to see whether or not the substance loses the hydrogen chloride easily. In case of acriflavine hydrochloride [G. W. Collins and A. Stasiak, "Comparative Chemical Examination of Different Brands of Acriflavine Hydrochloride (Acriflavine) and Acriflavine Base ('Neutral' Acriflavine)," JOUR. A. PH. A., 18, 659, No. 7, July 1929], it was found that practically all the hydrogen chloride could be removed by "drying" at 100° C.

TABLE II.—COMPARATIVE ANALYSES.

	Theory.[1]	Per Cent Found by A. M. A. Laboratory.[2] Mallophene.	Pyridium.
Loss over sulphuric acid		0.53	0.21
Loss at 100° C.		1.46	0.21
Water-insoluble material		0.20	0.14
Ash		0.27	0.08
Nitrogen (N^{---})	28.05	28.55	29.31
Chlorine (Cl^-)	14.20	14.96	14.18
Base,[3] $C_6H_5N:NC_5H_2N(NH_2)_2$	85.37	80.88	83.05
Hydrogen chloride (HCl)			
1. By calculation from chlorine (Cl^-)	14.61	15.38	14.58
2. By precipitation and subsequent titration of the filtrate[4]		15.47	14.92
3. Electrometric titrations			
(*a*) Undried specimen		15.45	14.84
(*b*) Specimen previously dried over sulphuric acid		14.74	14.50
(*c*) Specimen previously dried at 100° C.		14.38	14.49

[1] Calculated on anhydrous basis.

[2] All figures, except moisture or otherwise indicated are calculated to the dried basis.

[3] The base, phenylazo-alpha-alpha-diaminopyridine, was determined as follows: A weighed quantity (0.13 Gm.) of each of the specimens was transferred to a 250-cc. beaker and dissolved in 50 cc. of luke-warm water; with constant stirring, an excess of ammonia water was added; the beaker and contents cooled to about 0° C., and allowed to remain at that temperature for about one hour. The precipitate of the base, phenylazo-alpha-alpha-diaminopyridine, was collected on a Gooch crucible, washed with ice-cold water, dried at 100° C. and weighed. The method was considered unreliable, yielding only approximate results.

[4] The method of procedure: A weighed quantity (about 0.5 Gm.) of each of the specimens was transferred to a 500-cc. Erlenmeyer flask, and dissolved in 250 cc. of luke-warm water (previously boiled to remove carbon dioxide); with constant agitation, tenth-normal sodium hydroxide solution was added until neutralized (the base, phenylazo-alpha-alpha-diaminopyridine, was precipitated and the solution became yellow in color); followed by the addition of an excess of alkali (about 1 cc.), the mixture cooled to about 5° C. and allowed to stand for a few minutes. The separated base was removed by filtering through paper, collecting the filtrate and washing with cold water. Finally, the combined filtrate and washings were titrated with tenth-normal hydrochloric acid, using phenolphthalein as an indicator.

From the analytic data it was concluded that the formula $C_6H_5N:NC_5H_2N(NH_2)_2HCl$ phenylazo-α-α-diaminopyridine hydrochloride as given by the manufacturers is correct, and that the Mallinckrodt product appears to be essentially identical chemically with the Merck & Co., Inc., product, both containing minor impurities.

STANDARDS.

Based in part on the information in the literature and in part on the work reported herein, rigorous standards for identity and purity of phenylazo-α-α-diaminopyridine hydrochloride were elaborated. These were sent to Merck & Co., Inc., distributor, for comment and then, with certain slight modifications suggested by them, the following standards were adopted by the Council on Pharmacy and Chemistry for inclusion in "New and Non-official Remedies:"

Pyridium.—Phenylazo-2-6-diamino-pyridine Monohydrochloride.—Phenylazo-α-α-diaminopyridine monohydrochloride. $C_6H_5N:NC_5H_2N(NH_2)_2(2)(6)HCl$. The

monohydrochloride of an azo dye of the pyridine series, phenylazo-α-α-diamino-pyridine. Pyridium contains 28 per cent of nitrogen.

Actions and Uses.—Pyridium has marked penetrating power and is non-toxic and non-irritant in therapeutic dosage. It is rapidly eliminated through the urinary tract. It is bactericidal in aqueous solution against staphylococcus, streptococcus, gonococcus, *B. coli* and even *B. diphtheriæ*. It is proposed for use in gonorrheal infections, urinary diseases and in colon bacillus and mixed infections. In addition to its oral administration it is proposed for irrigation and other local application on the basis of its lack of harmfulness and of its bactericidal activity.

Dosage.—Pyridium is usually administered orally in doses of 0.2 Gm. (3 grains) three times daily either as powder in capsules or as tablets; for local application to wounds, ulcers and infected surfaces an aqueous solution of 0.5 to 1 per cent may be used; for urethral injection a 0.3 per cent solution is used.

Pyridium should not be used in connection with compounds containing mercury as it releases the mercury from such compounds. It is contraindicated in uremia and in severe disorders of the kidneys of a noninfectious type.

Pyridium is only slightly soluble in cold water, about 1 part in 300. However, it will form supersaturated solutions relatively easily. Pyridium will precipitate out of a 2 per cent (supersaturated) solution after approximately two days, it will precipitate out of a 1 per cent solution only after months. A 1 per cent solution may be stabilized for months by the addition of 10 per cent dextrose, U. S. P. X. Aqueous solutions should be prepared with distilled water, as the salts present in the tap water of many localities are liable to precipitate the free base, phenylazo-α-α-diamino pyridine.

Pyridium occurs as a red, odorless, microcrystalline powder, possessing a slightly bitter taste; slightly soluble in cold water, about 0.3 part in 100 parts, soluble in boiling water, about 5 parts in 100 parts; slightly soluble in alcohol, soluble in glacial acetic acid; insoluble in acetone, benzene, chloroform, ether and toluene, although these solvents might be colored slightly yellow by its presence, if the solvents are not entirely free from water; the aqueous solution (1 in 500) is red and is distinctly acid in reaction when measured by means of the hydrogen electrode. On addition of diluted sulphuric acid or a dilute solution of sulphates to an aqueous solution of pyridium, a precipitate forms.

Digest about 0.2 Gm. of pyridium with 10 cc. of diluted hydrochloric acid for thirty minutes at 50° C.; the dihydrochloride separates as small, dark red crystals or scales; decant the supernatant liquid and add 20 cc. of water to the crystalline precipitate; the material dissolves. Dissolve about 0.5 Gm. of pyridium in 500 cc. of water; add an excess of ammonia water; collect the resultant free base, phenylazo-alpha-alpha-diaminopyridine on a filter paper, wash, recrystallize from water and dry at 100° C.; it melts at 137° to 139° C.

Dry about 1 Gm. of pyridium, accurately weighed to constant weight over sulphuric acid in a partial vacuum; the loss in weight does not exceed 1 per cent. Incinerate about 1 Gm., accurately weighed, previously dried over sulphuric acid; the ash does not exceed 0.3 per cent. Dissolve about 1 Gm. of pyridium, accurately weighed, previously dried over sulphuric acid, in 200 cc. of warm water; collect the insoluble matter, if any, in a weighed Gooch crucible; wash the insoluble matter with hot water, dry the residue at 100° C. and weigh; the insoluble matter does not exceed 0.5 per cent. Determine the nitrogen content according to the Dumas method in Clarke's "Handbook of Organic Analysis," 2nd edition, page 199; the nitrogen is not less than 28.0 per cent nor more than 29.3 per cent, when calculated to the dried substance. Transfer about 0.5 Gm. of pyridium, accurately weighed, to a 600-cc. beaker, and dissolve in 250 cc. of water, previously boiled to remove carbon dioxide; the hydrogen chloride content determined

by titration with tenth-normal sodium hydroxide solution using the hydrogen electrode and a calomel cell (normal potassium chloride solution) corresponds to not less than 14.5 per cent nor more than 15 per cent of hydrogen chloride, when calculated to the dried substance.

SUMMARY.

1. Comparative chemical analyses were made of the two different brands of phenylazo-alpha-alpha-diaminopyridine hydrochloride appearing under different proprietary names.

2. Crystallographic examinations indicated: (1) that the second specimen of Pyridium was of a purer quality than the first one; (2) that all specimens appeared to contain a relatively small amount of an impurity; (3) that the second specimen of Pyridium and the specimen of Mallophene were essentially the same quality.

3. Both products contained varying amounts of moisture.

4. The p_H curves of the electrometric titrations proved to be a good criterion in the determination of the identity and purity of the compound. Furthermore, the electrometric titration demonstrates that when the hydrogen chloride was given off on exposure at 100° C., it was only the small quantity in excess of the stoichiometrical amount for a monohydrochloride.

5. The results obtained indicate a slight difference in purity; the Mallinckrodt product contained an excess of hydrogen chloride. The product of Merck & Co., Inc., was satisfactory in hydrogen chloride content but ran high in nitrogen content, indicating probably an impurity, such as an amide, which was not completely removed in the process of manufacture. On the whole, however, it was sufficiently pure for a product of this character.

6. The specimens of Mallophene and Pyridium examined contained essentially the same chemical compound.

7. Standards for phenylazo-α-α-diaminopyridine hydrochloride have been prepared, based on the work herein reported.

FLUECKIGERIANA.

BY EDWARD KREMERS.

V. Flueckiger letters to Power, 1882–1890.

(*Continued from p. 253.*)

Strassburg, Feb. 7, 1886.

MY DEAR FRIEND,

I am pleased with your good news of Jan. 19th and may say that I have already written long ago to the Berlin publisher, Mr. Springer, on account of the translation of the "Grundlagen" (1). So Messrs. Wood & Co. may fairly apply to him for the cliches (2).

As to the "Pharmaceutische Chemie" I have done with the first part, which as you will be well aware is by far the more difficult; so I hope to finish the work toward the end of this year or at least in the very first weeks of 1887. To give the preference to Messrs. Wood for the translation of the Pharm. Chemie I have no objection, still I suppose they will pay the translator. I believe it would be possible to obtain this.

Dr. Tschirch must be styled Lecturer of Botany and Materia medica in the University of Berlin, for he is simply "Privatdocent" (3) and not yet a professor.