(5) Part of these were from solution freshly made up, and part from solution made up September, 1911, and part from solution of June, 1910 (cf. table of permanency.)

The value .000001 gm. per gm. of frog for the M. L. D. is the mean of the first year's lethal doses for crystalline Kombe strophanthin. If the lethal dose of the standard tincture is taken at .000075 cc. per gm. of frog for the mean M. L. D. of the year, the toxicity of crystalline Kombe strophanthin comes out almost exactly .0000010 gm. per gm. of frog.

The value .000075 cc. for the average lethal dose for this tincture of Kombe Strophanthus is based on the average of a very large number of tests, extending over a number of years. It was the standard previously used for determining the strength of all the heart tonics manufactured by Parke, Davis & Co.

It seems established that the mean M. L. D. for crystalline Kombe strophanthin is .0000010 gm. per gm. of frog (Rana Pipiens of 10 to 30 gm.)

Other methods for determining the physiologic value of heart tonics were compared on this crystalline Kombe strophanthin.

The time necessary for a frog's heart to cease beating has been proposed by Focke[86] as a method for comparing heart tonic preparations.

He endeavors to determine the value of a preparation by the relation $V = \frac{P}{D.T.}$ where P is the weight of the frog, D is the dose of the material, and T is the time elapsing before complete cessation of the ventricle beat occurs.

(To be continued)

PHYTOCHEMICAL NOTES.

EDWARD KREMERS, MADISON, WIS.

Introduction.

It is almost twenty-five years ago that the writer was initiated into the realms of chemical research by his teacher and friend, Dr. Frederick B. Power, who was the first professor of pharmacy at the University of Wisconsin and who has contributed so much to plant chemical research, especially during the past ten years as director of the Wellcome Research Laboratory in London. This first experience acquainted the writer at the same time with the charm that is associated with the application of chemistry to the study of plant life, a charm that has never deserted him, though at times the pressure of other investigations and manifold duties compelled him to abandon phytochemical problems. That he has ever recurred to these problems becomes apparent, however, from the accompanying list of published notes, many of which are but fragmentary accounts.

The object of this compilation of titles is not so much to effect an inventory as it is to place in the hands of student investigators of this laboratory a con-

[86] Arch. d. Pharm. Bd. 241, p. 128. Ibid. Bd. 248, p. 345-76.

venient bibliography of the work done here, and, more particularly, to point out to them the connecting thought that makes many of these notes the result of a deliberate plan rather than of a haphazard seeking for new isolated facts.

This list does not include the titles of articles of purely chemical investigations of plant products, such as menthol and its derivatives, citronellal and its derivatives, abietic acid, etc., some of which have grown out of phytochemical investigations. Neither are the earlier investigations of Prof. Power nor more recent investigations of Prof. R. Fischer on alkaloids included. The catalogue, therefore, is not one of phytochemical investigations of the School of Pharmacy of the University of Wisconsin, but one of the fragmentary notes published by the writer or by students working under his directions.

The first plant investigated was the white ash, a subject suggested by Prof. Power for a graduation thesis (No. 1). The genetic relationship between *Fraxinus americana* from Wisconsin and the manna-yielding *F. ornus* from the Mediterranean suggested the examination of other species of *Fraxinus* that were available in this state. As a result *F. cambucifolia* and *F. viridis* were made subjects for graduation theses by students (Nos. 23 and 19). Whereas mannitol was found in some species, its stereochemical isomer, dulcitol, was found in others. The study of these species was not restricted, however, to the isolation of the hexatomic alcohols mentioned, but their closely related oxidation products, the sugars, and the glucosides were likewise investigated so far as time and material permitted. The study of the several species of *Fraxinus* promises to be fruitful of good results and should be continued, even though the collection of suitable material is difficult at times. See also Nos. 11 and 20.

The writer's first experience with volatile oils was obtained in connection with the oils of pennyroyal (No. 2), and citronella (No. 3), both oils being investigated at the suggestion of Professor Power and under his direction during the academic year 1886-'87. The contribution of the two papers to the American Pharmaceutical Association at the Cincinnati meeting in 1887 caused the writer to join the association at that time. The papers being read by Professor Lloyd, later brought the writer into touch with one who had done much for the chemical study of American medicinal plants when few realized the importance of this subject or the opportunities in this large, uncultivated field. Even today this is largely virgin soil waiting for the pioneer investigator.

The first article on pennyroyal was followed by several others (Nos. 4, 6, 14, 17), which, however, accomplished little more than to establish the presence of pulegone in this oil. The presence of pulegone was later established in two other oils of the mint family, namely: *Pycnanthemum lanceolatum* (Nos. 32 and 40), and *Mentha canadensis* (No. 39).

The initial study of citronella oil (No. 3) led to further studies (No. 13), principally, however, of the aldehyde citronellal, which are not enumerated in the list because they are not essentially phytochemical in character, but purely chemical.

Thus initiated into the study of an exceedingly attractive group of plant products, the writer has spent more time on the volatile oils than on any other subject. Among the oils first examined were some from the family of conifers: *Abies balsamea* (Canada balsam, Nos. 5, 25), *Pinus strobus* (leaves, No. 8),

Pinus palustris (No. 27), *Picea nigra* (No. 28), *Pinus cubensis* (No. 29), *Tsuga canadensis* (No. 33), *Picca alba* (No. 42), *Pinus sabiniana* (Nos. 45 and 66), an unknown species yielding Oregon balsam (No. 56), *Juniperus sabina* (No. 57), *Larix europaea* (No. 58), *Abies amabilis* of the Pacific coast (No. 59), *Pinus longifolia* of India (No. 63), *Pinus murryana, Picea Engelmanni, Pinus edulis,* and *Pinus flexulis,* all collected by Professor Pammel in Colorado (No. 71). These investigations represent the study not only of oils obtained from the oleoresinous exudations, but also of oils from leaves and cones. Thus, e. g., one of the leaf oils (hemlock) was found to be very rich in bornyl acetate, and, in a rational system of forestry, might be made a source for camphor.

The study of these species was not, however, restricted to their volatile constituents. The very first species enumerated above suggested the examination of the resin acids as well, which have long attracted the attention of chemists. As a result the resin acids from other species were included. Not only were the natural resin acids studied, but also such that had undergone changes in the process of preparation (Nos. 7, 9, 10, 12, 13). These phytochemical studies of the resin acids led, likewise, to the purely chemical study of abietic acid, not enumerated, in which, among other experiments, the first molecular determinations of abietic acid were made as a contribution to the much controverted subject of the size of the molecule of this acid. Other resins and oleoresins examined are those of an unknown species yielding Oregon balsam (No. 56), of *Larix Europaea* (No. 58), *Abies amabilis* (No. 59), *Pinus longfolia* (No. 63), *and Pinus sabiniana* (No. 66).

Attention has already been called to two local species of the mint family, *Pycanthemum lanceolatum* (Nos. 32 and 40), and *Mentha canadensis* (No. 39), which have been examined in addition to pennyroyal, *Hedeoma pulegioides* (Nos. 2, 4, 6, 14 and 17). Other labiate oils examined are those of spearmint, *Mentha viridis* (No. 16), peppermint oil, *Mentha piperita* (No. 18), *Mentha citrata* (No. 73), and the monarda oils.

The study of Monardas was begun in 1895 with the examination of the oil of the local *Monarda fistulosa,* a study that has been continued up to the present time (Nos. 26, 34, 36, 44, 47, 48, 51, 52, 55, 69, 75). It was extended by including *M. punctata* (Nos. 35, 43), growing on the more sandy soil of Arena, west of Madison, and has thus far included *Monarda didyma* (No. 50), from the East, and *Monarda citriodora* (No. 53, 77), from the South. A mere beginning has been made with a fifth species, viz., the *M. bradburiana.* As rapidly as other material can be secured, some of which is now being cultivated on university grounds, more species will be included within the scope of the investigation.

From its inception, the work on the Monardas was a chemical study in comparative plant physiology. As such it has proven exceedingly interesting. Not only, however, have the constituents of different species been compared, but the influence of cultivation and different seasons has been looked into. Moreover, and this is possibly the most important aspect of this study, the volatile constituents have not been studied merely by themselves, but in connection with the other constituents of the plants. Thus, e. g., it has been shown that at least some of the pigments of the monardas stand in direct relation to some of the volatile constituents, and that the ferments contained in the plant exert their influence

on both (Nos. 54, 55 and 69). In order to explain the different shades of color of the same pigment a study of the mineral constituents has been undertaken. For the purpose of taking stock, as it were, of the material accumulated in the study of the Monardas, a monograph has been prepared by Miss Nellie Wakeman and published as a bulletin by the University of Wisconsin. However, the work along this line is not to end here. Exact moisture and oil determinations have been made during the summer with cultivated material of *Monarda fistulosa.* A Missouri variety of *M. fistulosa* is being cultivated and a first sample of oil therefrom has already been distilled. Through the kindness of Professor Eberle, a large amount of *Monarda citriodora* has been obtained. It has been distilled and the volatile and pigment constituents are being examined. Further work on *Monarda punctata* is also under way.

Here again, as in previous instances, the initial study of plant products has led to the purely chemical study of some of their constituents. Thus the derivatives of thymol and carvacrol, their oxidation to hydrothymoquinone and thymoquinone, monohydroxy thymoquinone, and dihydroxythymoquinone, all of which are found in Monarda oils, and the subtle characteristics of the thymoquinhydrone and other quinhydrones and phenoquinones had to be studied by themselves in order to be able to interpret properly the observations made in connection with the plants. Some of these investigations have been reported on, but are not included in the phytochemical list; others are still in progress. The more general application of the quinhydrone hypothesis of pigmentation, which resulted from the study of the *Monardas,* is now being made the subject of a special study.

Among other oils examined are those of *Canella alba* (Nos. 15, 24), *Erigeron canadense* (Nos. 21, 30, 60, 65), Saw pelmetto (No. 46), *Pseudocymopterus anisatus* (No. 49), wintergreen and birch (No. 61), several new Artemesia oils (Nos. 62 and 64), American wormseed oil (No. 65*), oils from milfoil (No. 67), California eucalyptus oils (No. 68), celery seed oil (No. 74), wormwood oil (No. 78). The occurrence of methyl salicylate in nature is discussed in No. 41; the absence of hydrocyanic acid in *Mitchella repens* is shown in No. 38.

Triticum repens (No. 22), Kava kava (No. 31), Johore gambier (No. 37), the Johore products of the cocoanut palm (No. 72', and currant wine (No. 76), were likewise investigated. The reasons for taking up these several subjects need not here be mentioned; some have grown out of other work, others were examined upon request, etc.

The reference to methyl salicylate suggests calling attention to another piece of work that has grown out of the work on the volatile oils, but which does not strictly come within the scope of these "Phytochemical Notes." Reference is here had to the classification of the volatile constituents of plants in accordance with rational principles of modern classification and a critical discussion of their occurrence in the vegetable kingdom. The publication of this work was begun some years ago, but had to be interrupted temporarily on account of U. S. P. revision duties. This work has led not only to the classification of other groups of plant constituents, such as glucosides and alkaloids, but to a revision of the entire field of the classification of carbon compounds.

The writer trusts that these brief remarks on work scattered over a period of twenty-five years may throw some light on more than seventy fragmentary pub-

lications that must largely appear without connection or positive plan to the casual reader.

CHRONOLOGICAL LIST.

1886.

1. Edward Kremers. Analysis of the bark of *Fraxinus americana* Lin. Contributions from the department of Pharmacy of the University of Wisconsin, No. 2, p. 19.

1887.

2. Edward Kremers. Analysis of the volatile oil of *Hedeoma pulegioides.* Proc. A. Ph. A., 35, p. 546.
3. Examination of oil of *Andropogon nardus.* Proc. A. Ph. A., 35, p. 562.

1888.

4. Edward Kremers. Remarks of the analysis of the volatile oil of *Hedeoma pulegioides.* Pharm. Rundschau, 6, p. 103.

1891.

5. Gustave V. Kradwell. *Terebinthina canadensis,* with a review of the different varieties of turpentines. Proc. Wis. Pharm. Assn., 12, p. 37.
6. Edward Kremers. The volatile oil of *Hedeoma pulegioides.* Persoon. Pharm. Rundschau, 9, p. 130.
7. John L. Mead. Examination of black pitch. Proc. Wis. Pharm. Assn., 12, p. 43.
8. Walter A. Trayser. Examination of the leaves of *Pinus strobus.* Lin., Proc. Wis. Pharm. Assn., 12, p. 41.

1892.

9. Otto Hackendahl. Resin acid from black pitch. Proc. Wis. Pharm. Assn., 13, p. 48.
10. Wm. G. Kuntz. Burgundy pitch. Proc. Wis. Pharm. Assoc., 13, p. 53.
11. Rudolph H. Mieding. Examination of the inner bark of *Fraxinus americana,* L., Proc. Wis. Pharm. Assn., 13, p. 80.
12. Henry A. Peters. Examination of the resin of Canada balsam. Proc. Wis. Pharm. Assn., 13, p. 45.
13. Leopold C. Urban. Citronellone. Proc. Wis. Pharm. Assn., 13, p. 61.
14. William C. F. Witte. Examination of *Oleum hedeomae.* Proc. Wis. Pharm. Assn., 13, p. 55.

1893.

15. Harold N. Bruun. The volatile oil of *Canella alba.* Proc. Wis. Pharm. Assn., 14, p. 36.
16. Walter F. Gilman. The terpenes of spearmint oil. Proc. Wis. Pharm. Assn., 14, p. 53.
17. Charles J. Habhegger. On the composition of American pennyroyal oil. Proc. Wis. Pharm. Assn., 14, p. 51.
18. Robert I. Halsey. The hydrocarbons of peppermint oil. Proc. Wis. Pharm. Assn., 14, p. 88.
19. Henry O. Hilfert. *Fraxinus viridis, Michx.* (Green Ash.) Ph. Rund., 11, p. 215; also Proc. Wis. Pharm. Assn., 14, p. 63.
20. Edward Kremers. Dulcitol. Pharm. Rundschau, 11. p. 158.
21. Fritz W. Meissner. Oil of *Erigeron canadense,* L. ,Proc. Wis. Pharm. Assn., 14, p. 65.
22. Harriet R. Pope. Chemical constituents of rhizome of *Triticum repens.* Proc. Wis. Pharm. Assn., 14, p. 96.
23. Willibald, J. Wehle. *Fraxinus sambucifolia* (Black Ash), Lam. Proc. Wis. Pharm. Assn., 14, p. 61.

1894.

24. Robert T. Williams. The volatile oil of *Canella alba.* Pharm. Rundschau, 12, p. 183.

1895.

25. H. L. Emmerich. The volatile oil from Canada balsam. Am. Journ. Pharm., 67, p. 135.
26. J. L. Mead and J. J. Brennan. On the chemical composition of the volatile oil from *Monarda fistulosa, Linne.* Pharm. Rundschau, 13, p. 207.
27. J. D. Heald. Oleoresin from *Pinus palustris.* Pharm. Rundschau, 13, p. 135.
28. C. G. Hunkel. Oil from *Picea nigra.* Pharm. Rundschau, 13, p. 135.
29. C. G. Hunkel. Oleoresin from *Pinus cubensis,* Gries. Pharm. Rundschau, 13, p. 136.
30. Carl G. Hunkel. Terpineol from Oil of *Erigeron canadense.* Pharm. Rundschau, 13, p. 137.

1896.

31. Arthur E. Bossingham. On the chemical composition of Kava Kava. Proc. W. P. A., 16, p. 53.
32. Washington G. Correll. Oil from *Pycanthemum lanceolatum,* Pursh. Ph. Rev., 14, p. 32.
33. Carl G. Hunkel. Oil from *Tsuga canadensis, Carriere.* (Hemlock Oil.) Ph. Rev., 14, p. 34.

34. E. J. Melzner. On the chemical composition of the oil of *Monarda fistulosa*. Ph. Rev., 14, p. 198; also Proc. A. Ph. A., 44, p. 242.
35. W. R. Schuman. On the chemical composition of the oil of *Monarda punctata*. Ph. Rev., 14, p. 223; also Proc. A. Ph. A., 44, p. 238.

1897.

36. E. J. Melzner. Phenol content of the oil of *Monarda fistulosa*. Ph. Rev., 15, p. 86.
37. William O. Richtmann. Johore gambier. Ph. Rev., 15, p. 27.

1898.

38. Richard Fischer. Test for hydrocyanic acid in *Mitchella repens*. Ph. Rev., 16, p. 98.
39. Florence M. Gage. *Mentha canadensis*. Ph. Rev., 16, p. 412.
40. F. W. Alden. *Pycnanthemum lanceolatum*, Pursh. Ph. Rev., 16, p. 414.
41. M. M. James. On the occurrence of methyl salicylate. Ph. Rev., 16, p. 100.

1899.

42. W. A. Trayser. Some coniferous volatile oils. I. Oil of White pine. Ph. Rev., 17, p. 506.
43. W. E. Henricks. *Monarda punctata*. Ph. Archives, 2, p. 73.
44. I. W. Brandel. *Monarda fistulosa*. Ph. Archives, 2, p. 76.

1900.

45. Edward Kremers. Notes on coniferous oils. II. Oil from *Pinus sabiniana*. Ph. Rev., 18, p. 165.
46. Oswald Schreiner. Saw palmetto oil. Ph. Rev., 18, p. 217.

1901.

47. I. W. Brandel. Thymoquinone in wild bergamont oil. Ph. Rev., 19, p. 200.
48. I. W. Brandel. Hydrothymoquinone in wild bergamont oil. Ph. Rev., 19, p. 244.

1902.

49. Irwin W. Brandel. The volatile oil from *Pseudocymopterus anisatus*. Ph. Rev., 20, p. 218.

1903.

50. I. W. Brandel. Oil of *Monarda didyma*. Ph. Rev., 21, p. 109.
51. J. J. Beck. Oil from the corolla of *Monarda fistulosa*. Ph. Rev., 21, p. 111.
52. I. W. Brandel. Oil from the leaves of *Monarda fistulosa*. Ph. Rev., 21, p. 113.

1904.

53. I. W. Brandel. The volatile oil from *Monarda citriodora*. Ph. Rev., 22, p. 153.
54. Frank Rabak. Oxydase from *Monarda fistulosa*. Ph. Rev., 22, p. 190.
55. D. B. Swingle. The thermal death points of *Monarda ferments*. Ph. Rev., 22, p. 193.
56. Frank Rabak. Oregon balsam. Ph. Rev., 22, p. 293.

1905.

57. E. F. Ziegelmann. Oil of savin. Ph. Rev., 23, p. 22.
58. Frank Rabak. Oleoresin from *Larix europaea*. Ph. Rev., 23, p. 44.
59. ———. Oleoresin from *Abies amabilis*. Ph. Rev., 23, p. 46.
60. ———. Oil of *Erigeron canadense*. Ph. Rev., 23, p. 81.
61. E. F. Ziegelmann. Oils of wintergreen and birch. Ph. Rev., 23, p. 83.
62. Frank Rabak. On several new Artemesia oils. Ph. Rev., 23, p. 128.
63. ———. On the oleoresin of *Pinus longifolia*. Ph. Rev., 23, p. 229.

1906.

64. Frank Rabak. Notes on several new Artemesia oils. II. Ph. Rev., 24, p. 324.
65. ———. Oil of *Erigeron canadense*. Ph. Rev., 24, p. 326.

1907.

*65. Edward Kremers. An experience with American wormseed oil. Ph. Rev., 25, p. 155.
66. Frank Rabak. Oleoresin of *Pinus sabiniana*. Ph. Rev., 25, p. 212.
67. Arthur Sievers. Oils from milfoil. Rev., 25, p. 215.

1908.

68. Edward Kremers. California eucalyptus oils. 26, p. 177.
69. Nellie Wakeman. Quantitative determination of oxydase in the leaves of *Monarda fistulosa*. Ph. Rev., 26, p. 314.

1909.

*70.
71. John Swenholt. Four pine-needle oils from Colorado. Midland-Review, 43, p. 611.

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*Owing to editorial errors, No. 65 appears twice, but no No. 70.

1910.
72. Wm. H. Kendell. Palm sugar, vinegar and copra oil. Midland-Review, 44, p. 78.
*73. R. C. Roark. Oil from Mentha citrata.
74. John Swenholt. Oil of celery seed. Midland-Review, 44, p. 220.
75. S. K. Suzuki. Hydrothymoquinone and oxidation products from Monarda fistulosa. Midland-Review, 44, p. 342.
76. W. H. Kendell. Currant wine. Midland-Review, 44, p. 478.
77. C. Lefebvre and N. Wakeman. Oil of Monarda citriodora. Midland-Review, 44, p. 526.
1912.
*78. R. C. Roark. An unusual oil of wormwood.

THE IMPROVEMENT OF MEDICINAL PLANTS.†

F. A. MILLER, INDIANAPOLIS.

Certain suggestions were made before the last meeting of the Academy for the possible improvement of valuable medicinal forms through the application of breeding methods. Some of these suggestions have been carried out during the past summer upon experimental plots of belladonna, henbane, stramonium, digitalis and cannabis. The results, though only tentative, are extremely encouraging, and indicate a means of obtaining not only greater yields of the resultings drugs, but better and more reliable medicinal products.

Belladonna has shown great uniformity in morphological characters, but considerable variability in the percentage of alkaloids in selected plants. In a comparatively small number this variation was found to be over 50 percent, or from 0.52 percent to 0.87 percent total alkaloids as found in the highest and lowest yielding individuals. Much has been said concerning the variation in total alkaloids as influenced by various conditions. In fact, some experimental work has been done upon the influence of such factors as food elements, light and shade, soils, meteorology, etc., upon the production of alkaloids and other active principles. It now seems apparent, however, that before such data can have any scientific bearing, or be utilized as a means of following the influence of given factors, uniform strains of the plants under investigation must first be obtained. This apparent necessity is due to the wide variations which have been found to exist between the individuals of a given group which have been grown under uniform conditions.

A group of individual plants varying over 50 percent when grown under uniform ecological conditions cannot be expected to behave uniformly when grown under varied conditions. Differences no greater than 50 percent have been reported as being due to certain external influences as affecting all plants upon a given area, while according to recent individual plant investigations, such an area might produce plants varying this much or more among themselves, and representing at the same time any possible mixture with reference to yield. It seems necessary, for this reason, to first obtain a strain of the form under investigation, the individuals of which will react uniformly to certain external conditions. To investigate this point, plants of known alkaloidal yield are being propa-

*These studies will appear in later issues of this publication.—EDITOR.

†Read before the Botanical Section, Indiana Academy of Science, Nov., 1912.