SOME CONSTITUENTS OF RAGWEED POLLEN.

BY FREDERICK W. HEYL.

With this report, the chemical investigation of ragweed pollen is completed.[1] The work described below was carried out upon the ether and alcohol extracts, which had been removed before making the protein extracts that were first studied.

The fatty acids in the ether extract include formic, acetic, valeric, lauric, an unsaturated acid, $C_{10}H_{18}O_2$, oleic, linoleic, palmitic and myristic acids.

The alcoholic extract contained a mixture of phosphatides; repeated purification indicating the presence of an acetone-insoluble lipin fraction. The resin precipitated by pouring the alcoholic extract into water amounted to about 6.2% (exclusive of fat). Nothing crystalline could be derived from it.

The water-soluble part of the alcohol extract, from which the yellow coloring matters had been precipitated with basic lead acetate solution, yielded the following substances: a trace of guanosin, adenine, betaine (1.0%) sucrose, and levulose. Glucosides are absent.

The application of a systematic scheme of plant analysis to ragweed pollen has therefore yielded about 27 well defined substances, but with the exception of the coloring substances and possibly the proteose obtained from the water soluble fraction, none of these substances appear to represent any chemical specialization in this cell.

EXPERIMENTAL PART.

A. Ether Extract of Pollen was prepared by macerating 800 Gm. (moisture = 5.2%) which had been thoroughly desiccated over sulphuric acid with four portions of anhydrous ether, each extract amounting to about three liters. The ether was removed and the residue was found to be completely soluble in low boiling petroleum ether. This extract weighed 89 Gm.[2] and is equivalent to 11.1% of the pollen.

This extract is free from phosphorus.

The ligroin was removed, the material dissolved in ether and extracted with dilute hydrochloric acid, and with solutions of ammonium carbonate, potassium carbonate, and potassium hydroxide, but nothing of interest was found in the slight quantities thus obtained by means of the first two solvents.

The extraction with potassium carbonate was small but it was subjected to a steam distillation after acidification and the potassium hydroxide solution which contained about 7.8 Gm. was treated similarly.

These steam distillates were joined. Titration of a small aliquot showed that they required 13.0 cc $N/2$ alkali for neutralization. The acid distillate was shaken with ether. The aqueous layer was almost neutralized by the addition of 8 cc $N/2$ alkali and concentrated to a small volume. The repeated additions of small portions of silver nitrate solution to the hot solution showed the presence of considerable formic acid because of the formation of blackened reduced precipitates. When at length the formic acid had been removed by heating with silver nitrate a crop of crystals of a white silver salt was obtained.

0.0473 Gm. gave 0.0293 Gm. Ag. Calc. for $C_2H_3O_2Ag$: Ag, 64.6. Found: Ag, 61.9%

[1] *J. A. C. S.*, 39, 1470 (1917); 41, 670 (1919); 41, 1285 (1919); 42, 1738 (1920); 44, 2283 (1922).

[2] A few small samples were added to this material before proceeding.

This material is essentially silver acetate, but small quantities of a higher acid are present.

The ether extract of the acid steam distillate was dried over anhydrous sodium sulphate and the solvent was removed. The residue required 3.2 cc $N/2$ alkali for neutralization. The addition of silver nitrate solution precipitated about 0.3 Gm. of a heavy silver salt.

0.1177 Gm. gave 0.0391 Gm. Ag. Calc. for $C_{12}H_{23}O_2Ag$: Ag, 35.1. Found: Ag, 33.2.

This appears to be a small quantity of lauric acid.

The residues in the distilling flask after steam distillation were shaken out with ether and esterified by the process of I. K. Phelps and M. A. Phelps.[1] The solution of the esters in ether was extracted with solutions of potassium carbonate, and of potassium hydroxide but these extracts yielded nothing of interest. The esters were distilled at 15 mm. into three fractions (1)[2] 130-166° (2) 166–200°, (3)[2] 200–250°. These were joined and saponified. The acids, amounting to 4.0 Gm. were added to the non-volatile acids used in making lead salts as described below.

The ethereal extract of the pollen which had been shaken with solutions of hydrochloric acid, ammonium carbonate, potassium carbonate and potassium hydroxide was evaporated to dryness, and the residue dissolved in 200 cc alcoholic potassium hydroxide and saponified by boiling for five hours on a reflux.

The alcohol was removed, and the residue diluted with 500 cc water. The unsaponifiable matter was extracted with ether. The ether solution was dried over anhydrous sodium sulphate and upon removing the solvent 51 Gm. of an orange colored oil was isolated. The examination of this fraction has been previously reported.[3]

Examination of the Volatile Fatty Acids.—The alkaline solution from which the unsaponifiable material had been extracted with ether was acidified with dilute sulphuric acid and subjected to a vigorous steam distillation. The distillate was neutralized with a solution of barium hydroxide (equivalent to 11.7 cc N alkali) and concentrated to a volume of about 100 cc when a barium salt separated out. This was filtered off, returned to a small distilling flask, decomposed with sulphuric acid, and recovered by steam distillation. This steam distillate amounting to 100 cc was shaken with ether, and all the acid was thus extracted. The ether was removed and the residue almost exactly neutralized with 2.3 cc $N/2$ alkali and the silver salt prepared in the usual manner. It separated as a cheesy precipitate.

I, 0.2823 Gm. gave 0.1229 Gm. Ag. Calc. for $C_{10}H_{17}O_2Ag$: Ag; 39.0. Found. Ag, 40.0.

The neutral solution of the barium salts from which this had separated, contained considerable quantities of formic acid which was removed by boiling with silver nitrate solution. After filtering off the blackened precipitate there was obtained a series of silver salts amounting in all to about 0.6 Gm. The fourth was the heaviest fraction.

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[1] *Amer. J. Sci.*, (IV) **24,** 194–196 (1907).

[2] Iodine No. (1), 29.7; (3), 121.3.

[3] *J. A. C. S.*, **44,** 2283 (1922).

II 0.1398 Gm. gave 0.0586 Gm. Ag. Found 41.9% Ag
III 0.1986 Gm. gave 0.0918 Gm. Ag. Found 46.2% Ag
IV 0.0958 Gm. gave 0.0472 Gm. Ag. Found 49.3% Ag
V 0.0592 Gm. gave 0.0299 Gm. Ag. Found 50.5% Ag

Calc. for $C_{12}H_{23}O_2Ag$: Ag, 35.1; $C_6H_{11}O_2Ag$: Ag, 48.4.
Calc. for $C_{10}H_{17}O_2Ag$: Ag, 39.0; $C_6H_9O_2Ag$: Ag, 51.7.
Calc. for $C_8H_{15}O_2Ag$: Ag, 43.0.

The volatile acids appear to be a mixture consisting of formic, acetic, valeric, with higher ones probably lauric, and an unsaturated acid $C_{10}H_{18}O_2$. Whether any of the intermediate members of this series (caproic, caprylic and capric) are present, it is impossible to state.

Examination of The Non-Volatile Fatty Acids.—The acid mixture which had been steam distilled as above described was cooled and extracted with ether. From the dried solution the ether was removed and a residue of fatty acids weighing 28 Gm. was obtained. It was distilled under diminished pressure (15 mm.) and five fractions made: (I) boiling at 130–178°; (II) 178–198°; (III) 197–240°; (IV) 240–258°; (V) 258–265°. These amounted to 1.8; 4.0; 15.3; 3.4; and 1.5 Gm. respectively. The upper three fractions were united and found to have an iodine number of 60.6.

The presence of a small amount of low boiling acids in fractions (I) and (II) indicated the incomplete removal of volatile acids in the previous steam distillation or rather the presence of acids whose volatility lies midway between the two distinct groups.

The low boiling acids were twice systematically distilled and four fractions made (15 mm.).

Fraction (I) (B. p. up to 153°) very small quantity.[1]

Fraction (II) (B. p. 153–159°). This fraction was collected with reference to the boiling point of capric acid, but analysis indicated that the acid is not $C_{10}H_{20}O_2$.

Calc. for $C_{10}H_{20}O_2$: C, 69.8; H, 11.6.
Found: C, 71.0; H, 10.9.

This fraction when dissolved in aqueous solution of sodium carbonate instantly reduced potassium permanganate solution in the cold and the presence of a quantity of unsaturated acid is thus shown.

Fraction (III) (B. p. 159–170°). This was likewise unsaturated.

0.2622 Gm. subst. required 27.78 cc *N*/20 alkali for neutralization. M. wt., 188.7.
Calc. for $C_{10}H_{18}O_2$, 170; for $C_{12}H_{24}O_2$, 200.

Fraction (IV) (B. p. 170–179°). Analyzed as follows:

Calc. for $C_{10}H_{18}O_2$: C, 70.6; H, 10.6; Iodine No., 149.4.
Calc. for $C_{12}H_{24}O_2$: C, 72.0; H, 12.0.
Found C, 71.0, 71.2; H, 10.9, 11.0. Iodine No., 109.

This appears to correspond best to a mixture of 73% $C_{10}H_{18}O_2$ and 27% of $C_{12}H_{24}O_2$ (Lauric acid).

The higher boiling fractions were united with the acids that occurred free in the pollen and a quantity amounting to 25 Gm. was converted into the lead salts,

[1] Indicates lack of capric, caprylic and caproic acids.

which were dried and digested with ether. The lead salt soluble in ether yielded 9.2 Gm. liquid acids amounting to 36.8%. These were distilled at 15 mm. pressure and four fractions collected. (1) 148–200°; (2) 200–220°; (3) 220–241°; (4) 241–270°. The iodine numbers were (1) 101.3; (2) 92.4; (3) 144.7; (4) 140.0.

The third fraction which represented most of the liquid acids distilled chiefly at 228–238° and when analysed gave values corresponding with those required by a mixture of oleic and linoleic acids.

Calc. for $C_{18}H_{34}O_2$: C, 76.6; H, 12.1; Iodine No., 90.1.
Calc. for $C_{18}H_{32}O_2$: C, 77.1; H, 11.4; Iodine No., 181.4.
Found C, 76.9; H, 11.6.

The solid acids prepared from the lead salts insoluble in ether, which amounted to about 15 Gm. showed an iodine number of 11.8. These were dissolved in absolute alcohol and the solution gradually concentrated but no separation of the more insoluble fatty acids took place. The first fraction separated from quite concentrated solution, melted at 52–54°, and appeared to be impure palmitic acid, but there was no indication of the presence of stearic acid.

Calc. for $C_{16}H_{32}O_2$: C, 75.0; H, 12.5; N. V., 219.1.
Calc. for $C_{18}H_{36}O_2$: C, 76.1; H, 12.7; N. V., 197.5.
Found C, 74.6; H, 12.5; N. V., 203.9.

The presence of lower members of the series is evident. The acids were therefore esterified and distilled and a quantity of the esters distilled over below 180° at 20 mm. pressure. The free acids prepared from this fraction melted at 38–41°. The main fraction boiled at 180–200° which indicates a fraction consisting essentially of palmitic ester but containing nevertheless some lower members.

The fraction having the boiling point of palmitic ester (200–205°) was saponified. The acid melted at 50–52°, and upon analysis gave values agreeing closely with palmitic acid. (C, 75.0; H, 12.6; N.V., 207.)

The fraction boiling at 205–210° upon saponification gave an acid melting at 51–53°, and indicates perhaps the presence of a small amount of stearic acid.

The quantity of ester distilling above 210° was small. The solid acids obtained consist therefore of a mixture of palmitic with lauric, and very probably myristic acid and a trace of stearic acids.

B. THE ALCOHOL EXTRACT.

After exhausting with ether, the pollen (1150 Gm.) was percolated with alcohol and the percolate was concentrated to a volume of 3150 cc.

Pollen Phosphatide.—An aliquot (605 cc) was concentrated to a volume of 60 cc and completely precipitated by the addition of 500 cc of ether. A heavy yellow precipitate separated. The clear yellow supernatant solution was decanted and the ether was removed in a vacuum. The residue was treated with 200 cc of ether, and after 24 hours the mixture was filtered. The clear filtrate was again concentrated and redissolved in about 200 cc absolute ether and a trace proved insoluble. The ether solution was clarified by centrifugation and then washed with water, and dried over sodium sulfate. The dried solution was concentrated to a volume of 10–20 cc and mixed with acetone (400 cc). On standing and centrifugation the phosphatide separated as a yellowish-brown butter-like mass.

The precipitate was dissolved in warm ether and the solution made quantitatively to 200 cc. (Slightly turbid.)

20 cc contained 0.0703 Gm. dissolved solids. 20 cc gave 0.1848 Gm. $PbMoO_4$ = 1.275 mg. P.

As this (0.006%) is less phosphorous than was calculated from the previous[1] determination, the acetone filtrate was examined, being made quantitatively to a volume of 500 cc.

25 cc gave 0.5508 Gm. dissolved solids. 25 cc gave 0.0662 Gm. $PbMoO_4$ = 0.457 mg. P.

The acetone precipitation therefore yielded a precipitate weighing 0.7 Gm. and containing 0.01275 Gm. P, while the filtrate contained 11.02 Gm. and contained 0.0091 Gm. P, a considerable loss of phosphatide having resulted.

The crude phosphatide therefore contained about 1.8% P.

This phosphatide was further examined. A quantity of the ether solution (0.35 Gm.) was refluxed for 8 hours with 50 cc of 1% sulphuric acid after removing the ether. The fatty acids were extracted three times with ether, and the yield was 0.2476 Gm. (70.7%). These had an iodine number of 107.4.

The acid hydrolysis liquid when examined by the Walker-Munson process gave 0.0204 Gm. Cu_2O = 8.46 mg. dextrose = 2.4% of the phosphatide.

0.2812 Gm. phosphatide required 2.8 cc $N/10$ acid by Kjeldahl. N, 1.4%.

This phosphatide belongs to the same class as those from the pollens of *Alnus viridis* and *Pinus montana* described by Winterstein and Hiestand.[2]

The Resin.—The main part of the alcoholic extract was not used for the preparation of the phosphatide. To this main part was added the many by-products from the above described preparation. The whole solution (4 l.) was concentrated further and then poured into water and as the separation was unsatisfactory, the remainder of the alcohol was distilled off. The aqueous layer was syphoned from between the floating oily resin and the precipitated resin. It required centrifugation. The resin was washed with water, the washings being added to the original water solution.

The resin was dissolved in alcohol and the solution was poured upon purified sawdust and thoroughly dried. It was extracted as follows:

Petroleum ether (40–60°)	43.6 Gm.
Ether	1.2
Chloroform	3.7
Ethyl Acetate	20.1
Alcohol	32.5
Total	101.1 Gm.

Petroleum Ether Extract.—This solution (43.6 Gm.) consisted chiefly of fat, and it was studied in parallel with the primary ether extract of pollen, with special reference to the unsaponifiable material. There was added to it 53 Gm. of the ether extract obtained from further quantities of pollen. This work has been previously reported.[3]

[1] 0.026 % P.

[2] *Z. physiol. Chem.*, 54, 228 (1908).

[3] *Loc. cit.*

The Ether Extract (1.2 Gm.) upon standing separated a small amount of colorless substance which was not phytosterolin but a resin acid of indefinite melting point. A part of it was titrated but the deep orange color interfered with the phenolphthalein end-point.

0.1012 in alcohol required 5.27 cc 0.05 N alkali. M. wt., if monobasic, 376.

It was hydrolysed with 10% sulphuric acid in dilute alcohol and was found to be not glucosidic, and nothing of interest resulted.

The ethereal filtrate was extracted with the usual alkaline solvents but yielded nothing except to 10% potassium hydroxide which extracted it almost completely. This product was a resin.

The Chloroform Extract (3.7 Gm.) separated a small quantity of a substance which proved to be exceedingly soluble in alcohol but uncrystallizable. It melted at approximately 100–120°.

The filtrate when extracted yielded most of its material to the potassium carbonate solution and upon acidifying only amorphous material separated.

The Ethyl Acetate Extract of the Resin (20.1 Gm.) upon concentration and prolonged standing separated 1.7 Gm. of a white poorly formed product that softened at 190° and decomposed at about 202° with effervescence. It was practically insoluble in boiling alcohol, pyridine or ethyl acetate and was therefore an alteration product of the nature of a phenolic resin being readily soluble in alkalies.

The clear yellow filtrate was further concentrated and a further indefinite separation gradually accumulated. The filtrate was subjected to an acid and an alkaline hydrolysis without positive results. It proved to be non-glucosidic.

The Alcoholic Extract of the Resin (32.5 Gm.) was concentrated and permitted to stand in a vacuum desiccator whereupon it failed to crystallize and went to a varnish. When this alcohol extract was boiled with water, the resin melted to an oil and floated upon the surface. Upon attempting to redissolve it in alcohol a quantity of an alteration product (1.0 Gm.) remained insoluble. It softened at 161° and decomposed at slightly higher temperatures. Upon concentrating 16.3 Gm. further separated that softened at 152° and effervesced at 170–180°. This was difficultly soluble in alcohol but failed to crystallize.

This material dissolved in alkali forming an intensely yellow solution (Acid No., 161–207; Saponification no., 246–271).

The alcoholic filtrate was boiled with a quantity of sulphuric acid in dilute alcohol (to make the solution 5%), but no evidence of the formation of sugars was found. Nothing crystalline was found in the reaction mixture.

Examination of Water Soluble Constituents of Alcoholic Extract.—The solution (5 l.) was concentrated under diminished pressure to about 500 cc and a small amount of resin separated. This was added to the main resin fraction.

The solution was ether extracted. The ether extract was shaken with solutions of ammonium carbonate, potassium carbonate and potassium hydroxide. The last solvent extracted 2–3 Gm. of a resin which had the appearance of the resin acid obtained from Sumbul.

The aqueous solution was now completely precipitated with lead subacetate solution and the yellow glucosidic coloring matters consisting chiefly of glucosides of quercitrin and isorhamnetin[1] were thus isolated.

[1] Loc. cit.

The filtrate from the yellow lead salts was divided into two equal parts and the first half was freed from lead with hydrogen sulphide and concentrated to a small volume and allowed to stand in a desiccator but nothing crystalline resulted.

The residue was taken up in 5% sulphuric acid and the bases precipitated as phosphotungstates in the usual manner. The bases were liberated by the method of Wechsler and finally obtained in a slightly acid (nitric) solution from which the purines were precipitated with silver nitrate.

These silver salts were digested with ammonia water and divided into two parts: (*a*) those soluble in ammonia and (*b*) purine silver salts, insoluble in ammonia.

The part soluble in ammonia could not be identified but very probably consisted of guanosin. The systematic examination[1] of (*b*) yielded 0.3 Gm. adenin picrate m. p. 277–282°.

The filtrate from the purine silver salts was examined by the method of Kossel and Patten, but there was not enough histidin or arginin present for identification. The histidin mercury fraction contained 12 mg. nitrogen and gave Pauly's test, while the insignificant arginin silver fraction contained only 4 mg. nitrogen.

The Lysin Fraction was reprecipitated with phosphotungstic acid and the bases recovered in the usual manner. The solution was concentrated to a small volume and diluted with alcohol. Upon subsequent concentration to 10–15 cc a colorless crystalline mass (5.1 Gm.) separated. It decomposed at 298°.

Calc. for $C_5H_{11}O_2N$: C, 51.4; H, 9.4.
Found C, 51.1; H, 9.4.

It is therefore evident that the nitrogenous constituent of pollen, soluble in alcohol is largely betaine.

The hydrochloride decomposed with effervescence at 236–238°. It was fractionally crystallized.

Calc. for $C_5H_{11}O_2N.HCl$: Cl, 23.1.
Found (I) 22.85; (III) 22.7.

When nothing could be further isolated by direct crystallization the filtrates were allowed to desiccate completely and were boiled with large volumes of absolute alcohol until practically the entire material dissolved. Magnificent crystals of sucrose separated that decomposed at about 188° and were optically active $[\alpha]_D = +66.5°$.

Calc. for $C_{12}H_{22}O_{11}$: C, 42.1, H, 64.
Found C, 42.15; H, 6.3.

Another portion of the filtrate from the lead salt ($^1/_4$) was freed from lead with hydrogen sulphide and the filtrate from the lead sulphide was concentrated (finally in the presence of $CaCO_3$) to a small volume and then aliquoted into three parts, equivalent respectively, to 4, 33.5, and 250 Gm. portions of pollen.

EXAMINATION OF SYRUP BY BOURQUELOT'S BIOCHEMICAL METHOD.[2]

Pollen was shown to contain sucrose (0.4%) and the good agreement of the methods indicates no interfering sugars. The directly reducing sugar is levulose which was present (0.5%); β glucosides are absent.

[1] Jones, "Nucleic Acids," page 95 (1914).
[2] *Archiv. d. Pharm.*, **245**, 172 (1907). "Handbuch d. Biochem. Arbeit," VII, 766.

The following analysis was accompanied by proper controls.

(*a*) *Direct.*—The syrup (equivalent to 250 Gm. Pollen) + 6.25 cc 0.5 *N* sodium phosphate solution + hydrochloric acid (final acidity = 0.001 *N*) and thymol water q.s. to 250 cc. Rotation in 2 dcm. tube at 26° = + 0.52° V. Walker-Munson process. 5 cc gave 0.0565 Gm. Cu_2O. Levulose, 0.5%.

(*b*) *Action of Invertase.*—200 cc of the above solution + 0.25 Gm. active invertase. Readings at 24 hour intervals in 2 dcm. tube at 26° is constant at —1.55° V. 5 cc gave 0.1024 Gm. Cu_2O. Sucrose by reduction is 0.39%. By Clerget's formula it was 0.40%.

(2) Calculated to normal solutions, the direct rotation is 0.135° V and the calculated rotation for sucrose is 0.4° V. Hence the other sugar is levorotatory.

(*c*) *Action of Emulsin.*—100 cc of the above solution was plunged into boiling water-bath for 10 minutes. The solution was cooled to room temperature and 0.5 Gm. active emulsion added. After 3 days no increase in the reducing power was observed. 5 cc gave 0.1032 Gm. Cu_2O. The reading, which was obscure in 2 dcm. tube at 27°, was —1° V.

Pentose sugars could not be detected. The remainder of this material (33.5 Gm.) was treated with phenylhydrazine hydrochloride and sodium acetate and a yield of d. phenylglucosazone (0.12 Gm.) melting at 208° was obtained.

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COLOR STANDARDIZATION IN THE U. S. PHARMACOPŒIA.*

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Color perception is due to the appreciation through the retina of light waves of differing wave lengths. The spectrum of white light is the physicist's basis of color study, but in nature colors are developed that are of greater intensity and clearness than the spectrum of white light displays.

The color of opaque or partially opaque objects is due to the absorption of all the color waves falling on the object, except those particular colors which are reflected from the object and can thus be received by the eye. The color of transparent objects, solid or liquid, results from the transmission through the object of those colors seen by the eye and the absorption of any others that may be present. The color of flaming gases is due to the actual production of certain color waves which may then be perceived by the retina. One can readily understand, therefore, that a color chart prepared with opaque pigments might be more valuable for determining the color of opaque objects than for matching the color of a transparent object or a flaming gas. This is true perhaps to a limited extent.

The three Primary colors—yellow, red and blue, as indicated in chart I[1] are called primary because they are the basis of all other colors. In the white light spectrum these three colors cannot be seen with the intenseness and clearness displayed by the pigments used in this chart. However, by the sodium flame it is possible to produce an intense yellow, by the lithium a brilliant red, etc.

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* Scientific Section A. Ph. A., Cleveland meeting, 1922.

[1] The charts were displayed during the reading of the paper.