

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WYOMING.]

SOME CONSTITUENTS OF THE LEAVES OF ZYGADENUS
INTERMEDIUS. III.

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In a recent communication¹ from this laboratory the isolation of a characteristic alkaloid "Zygadenine" was described. Since poisonous properties have also been ascribed to the resin of *Zygadenus venenosus*² a variety exceedingly similar to that here investigated, we have extended the chemical examination of the plant to other constituents, following some of the methods which have been elaborated at the Wellcome Research Laboratories, London.

We have, however, been unable to isolate any toxic substances from the resin, and our conclusions concerning the resin, have been confirmed by a series of physiological tests³ made upon dogs. In these experiments, the resin, administered by mouth, proved to be physiologically inert.

Experimental.

The material used in this investigation was collected under the supervision of Professor Aven Nelson, during the latter part of May and the first week in June. The leaves weighed about 66 kilograms in the green condition, and corresponded to 16.5 kilograms of the dried material, upon which the extraction was conducted.

This material was extracted with 40 liters of 95% alcohol for seven days at a slightly elevated temperature, the maceration mixture being occasionally brought to the boiling point. The tincture was removed, and the partially exhausted plant was extracted with alcohol once more.

The combined extracts were concentrated under diminished pressure and when the volume had been reduced to approximately three liters the extract was poured into 15 liters of distilled water which had been acidified by the addition of 45 grams of tartaric acid. The precipitation of the resin at this dilution was complete. This resin by repeated shaking and long standing, was thoroughly extracted by the tartaric acid solution. By filtering off the resin, we obtained two fractions (1) the plant substances soluble in the aqueous tartaric acid, and (2) the resin, which was a black semi-solid.

Examination of the Tartaric Acid Solution.—This solution without concentration was completely extracted by shaking repeatedly with ether. The ether extracts were concentrated to a small volume and allowed to stand, but nothing crystalline separated. When all the ether had evaporated a

¹ THIS JOURNAL, 35, 258 (1913).

² Vejux Tyrode, *Biochem. Centr.*, 1904, Ref. No. 67.

³ Our thanks for these tests are due to Professor L. B. Mendel of the Sheffield Scientific School.

thick, black tar remained. When now, this was treated with a small volume of absolute ether, the black tar for the most part dissolved, leaving a yellow crystallin residue, insoluble in ether, which was filtered off with suction and washed with a small quantity of ether. This material weighed about 2 grams and after repeated crystallization from dilute alcohol it melted at 317° – 318° with decomposition. This melting point was not altered by further crystallization.

Calculated for $C_{15}H_{10}O_7$: C, 59.6; H, 3.3.

Found: C, 59.74; H, 3.62.

This substance agrees in its properties with *quercitin*, although the melting point is several degrees higher than any previously recorded.

The original ethereal filtrate from which the quercitin had separated was extracted successively with solutions of ammonium carbonate, sodium carbonate, and potassium hydroxide. The first solvent extracted 11.2 grams of a brown oil, but nothing could be isolated from it. The total weight of the material extracted by sodium carbonate was 8.6 grams, while the potassium hydroxide solution extracted practically nothing.

The ethereal solution was then washed with water, dried over sodium sulfate and evaporated to dryness. The residue was a light green oil of pungent odor in which a few crystals were suspended, but the amounts were very slight.

The tartaric acid solution, which had been extracted with ether, was now rendered alkaline with sodium carbonate, and extracted with ether. As the removal of the alkaloid progressed unsatisfactorily, some sodium hydroxide was added, and the extraction with ether continued. Eventually the extraction with ether was discontinued, and chloroform substituted. As described in a previous paper,¹ these extracts yielded a crystallin alkaloid. The extraction of alkaloid was, however, incomplete. The aqueous alkaline liquid was therefore rendered slightly acid with acetic acid, and concentrated under reduced pressure to a volume of 3.5 liters. It was now rendered alkaline and repeatedly extracted with amyl alcohol.

These amyl alcoholic extracts were united and concentrated somewhat and then extracted with aqueous tartaric acid solution. When these acid extracts were united and rendered alkaline with sodium carbonate 2.5 grams of amorphous alkaloidal material separated. This material was soluble in water, and in dilute hydrochloric acid. It precipitated as a white flocculent mass when the hydrochloric acid solution was rendered alkaline with ammonia. It gave a white, voluminous precipitate with Mayer's reagent. A precipitate was formed when a solution of iodine in potassium iodide or of picric acid was added to the solution of the base. Attempts to prepare this alkaloid in a crystallin condition failed.

¹ *Loc. cit.*

Physiologically, it failed to show any marked toxic properties for 0.10 gram introduced subcutaneously into a guinea pig was without effect.

The alkaline liquid was again rendered slightly acid with acetic acid and repeatedly shaken with hot amyl alcohol, about fifteen liters of amyl alcohol being used. The amyl alcohol was removed from the extract as far as possible by distillation under reduced pressure and the residue was digested with a large volume of petroleum ether. The material insoluble in the mixture of amyl alcohol and petroleum ether weighed 31 grams. Nothing crystallin could be obtained from it.

The filtrate from this material was examined further and a small quantity of sulfur (0.03 gram) was isolated. The presence of sulfur here can probably be accounted for, by the assumption that small quantities of thioether compounds were originally present in this plant, as their presence has been demonstrated in several of the bulbous plants among the *Liliaceae*.

The aqueous liquid which had been extracted with amyl alcohol, was subjected to a vigorous steam distillation to remove the dissolved amyl alcohol, diluted to 20 liters and precipitated with an excess of lead subacetate. This gave a voluminous yellow precipitate from which nothing was isolated.

The filtrate from the lead subacetate was freed from lead with hydrogen sulfide and the filtrate from the lead sulfide was concentrated to a heavy syrup under diminished pressure. This contained a very large quantity of sugar and yielded *d*-phenylglucosazone, melting at 210°. Analytical determinations previously published¹ showed that the leaves contain 5.6% sucrose, 5.89% reducing sugar, and 3.26% dextrin.

Examination of the Resin.—The air-dried resin weighed about 812 grams. This was dissolved in 5 liters of boiling 95% alcohol and upon cooling a considerable quantity of gelatinous material separated out. This was taken up in 700 cc. of 95% alcohol, and upon standing 65 grams (dried at 85°) again separated. The alcoholic solutions from which this material separated, were joined and an aliquot containing 375 grams was poured upon purified sawdust. The alcohol was removed and the residue dried at 85°.

The sawdust impregnated with resin was transferred to a continuous extractor. The less soluble resin was treated similarly. The results of the extractions were as follows:

	A.	B.
Ligroin extract.....	234.7 grams	55.7 grams
Ether extract.....	51.0 grams	5.2 grams
Chloroform extract.....	58.2 grams	1.2 grams
Ethyl acetate extract.....	5.3 grams	0.6 gram
Alcohol.....	20.4 grams	1.9 grams
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Total.....	369.6 grams	64.6 grams

¹ THIS JOURNAL, 33, 206 (1910).

The second resin proved to consist chiefly of fatty material and was not further examined.

The Ligroin Extract.—For the further examination of this material 196 grams were dissolved in about 2 liters of ether, and this solution was extracted successively with solutions of ammonium carbonate, sodium carbonate, and potassium hydroxide. During the extraction with ammonium carbonate a small amount of amorphous material separated, which was insoluble alike in ether and in the ammonium carbonate solution. When the ammonium carbonate extract was acidified 6.5 grams of amorphous material separated. Acidification of the sodium carbonate extract precipitated 0.45 gram of amorphous brown material.

The potassium hydroxide extract contained in suspension 15 grams of amorphous material. The extract was acidified and the precipitated fatty acids extracted with ether. The ether extract was filtered, dried over anhydrous sodium sulfate, concentrated to a small bulk, and 1.5 liters of petroleum ether added. A heavy black tar weighing 5 grams separated out and was removed by filtration.

The solvent was removed and the residue distilled under diminished pressure. The total weight of distillable acids was 28 grams (239° – 260° at 25–30 mm. Iodine number = 115.2). It was observed that above this temperature the acids solidified in the tube and a last fraction was therefore made of this material. After repeated recrystallization from ethyl acetate the melting point of this fraction remained sharply and constantly 75° .

Calculated for $C_{26}H_{52}O_2$: C, 78.8; H, 13.1.
Found: C, 78.2; H, 13.0.

This material would therefore appear to be cerotic acid.

The acids were now converted into their lead salts and the liquid acids separated from those of the saturated series in the usual manner. The liquid acids amounted to 62.6% of the total acids.

The solid acids were regenerated from the corresponding lead salts and joined to the solid acids isolated after saponification of the fat as described below.

The liquid acids were again distilled. The greater portion distilled at 220° – 254° at 17 mm. and a second fraction was collected from 254° up.

Fraction I. 0.1534 gram subst. absorbed 0.2807 gram iodine; iodine No. = 183.

Fraction II. 0.1626 gram subst. absorbed 0.2794 gram iodine; iodine No. 171.8.

$C_{18}H_{34}O_2$, $C_{18}H_{32}O_2$, $C_{18}H_{30}O_2$; iodine value = 90.1, 181.4, and 274.0 respectively.

The ethereal solution which had been extracted as described above was evaporated to dryness and the residue was saponified by boiling with alcoholic potassium hydroxide. The alcohol was then removed and water added. Some of the unsaponifiable matter separated as a brown flocculent solid. This was now extracted repeatedly with a large volume of ether,

which extracted the unsaponifiable products of the fat, and then the alkaline liquid was acidified with sulfuric acid and the liberated fatty acids were extracted with ether.

The Fatty Acids.—This ethereal solution was dried over anhydrous sodium sulfate and concentrated to a small bulk, and a large volume of ligroin was added. Nine grams of amorphous matter were filtered off, and the filtrate after removal of the solvent was distilled. It boiled at 216° – 265° at 16 mm. (iodine number 119.8) and a small fraction collected above this temperature yielded cerotic acid after repeated recrystallization from ethyl acetate. The yield of fatty acids weighed 19.4 grams.

This material was converted into the lead salts and the liquid acids separated from the solid acids in the usual manner. The liquid acids corresponded to 63.5% of the total weight, and had an iodine number of 162.

In order to gain more accurate information concerning the liquid acids 10.7 grams of mixed material were oxidized with a 1.5% solution of potassium permanganate.¹ From the oxidation mixture there was isolated a very small quantity of dihydroxystearic acid, which crystallized from alcohol in laminae that melted at 126° . The chief product was sativic acid. This crystallized from a large volume of water in microscopic needles that melted at 152° – 153° and weighed about 0.8 gram. This material required several recrystallizations from alcohol before it approximated purity.

Calculated for $C_{18}H_{36}O_4$: C, 62.1; H, 10.3.

Found: C, 61.6; H, 10.2.

In addition a quantity of isolinusic acid (172° – 175°) was isolated. The liquid acids therefore consist of a mixture of linolic, oleic, and isolinolenic acids, the first greatly preponderating.

The Solid Acids.—The solid acids derived from the hydrolysis of the glycerides were united with those that occurred free in the fat and these were subjected to fractional crystallization from alcohol. Of the three fractions, the first two were analyzed and the results indicated that an acid lower in the series, than palmitic was present and gave no indication of stearic acid.

Calculated for $C_{18}H_{32}O_2$: C, 75.0; H, 12.5. N. V. = 219.1.

Calculated for $C_{18}H_{30}O_2$: C, 76.1; H, 12.7. N. V. = 197.5.

Found Frac. I: C, 74.9; H, 12.53.

Found Frac. II: C, 74.56; H, 12.45.

The melting points of these fractions were 54° – 55° , 56° and 51° – 53° , respectively. Evidently no separations had been effected. The first two fractions were united and fractionally precipitated with a hot alcoholic solution of barium acetate in the usual manner. Six fractions were ob-

¹ Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," 1909, Vol. I, p. 458.

tained and the most insoluble fraction was again fractioned by the same method and divided into two parts. Thus we had seven fractions. Analytical data upon the first four of these are as follows:

I. Melting at 54° – 55° . C, 76.1; H, 12.81. 0.2033 gram neutralized 0.04246 gram KOH. Neutralization value = 208.8.

II. Melting at 54° – 56° . 0.3122 gram neutralized 0.06385 gram KOH. Neutralization value = 204.5.

III. Melting at 58° – 60° . C, 75.02; H, 12.69. 0.3050 gram neutralized 0.06508 gram KOH. Neutralization value = 213.4.

IV. Melting at 56° – 59° . 0.2994 gram neutralized 0.06347 gram KOH. Neutralization value = 212.0.

The solid acids consist of a mixture of stearic and palmitic acids and also of an acid of lower carbon content than palmitic, but the neutralization values indicate the predominance of palmitic acid.

Examination of the Unsaponifiable Matter.—The ether was removed from this fraction and an orange colored residue weighing 28 grams was obtained. This was viscid at first but upon cooling appeared partially crystallin. This material was dissolved in one liter of boiling absolute alcohol, and after prolonged standing 0.23 gram of material crystallized out, the melting point of which was about 62° – 63° . The filtrate was concentrated to a volume of 800 cc. and a very slight separation of the same material (0.04 gram) took place. The solution was now concentrated to 600 cc. and a very slight quantity again separated. Nothing further resulted from concentration until a volume of 200 cc. was arrived at when a slight separation of material melting at 134° – 135.5° took place. From the mother liquor about 2 grams more were isolated from very concentrated solutions.

The material which separated first was heated with phthalic anhydride, and a small quantity of an alcohol was removed in the usual manner.¹ The ether-chloroform solution was evaporated and the residue heated with alcoholic potassium hydroxide. This solution upon the addition of dilute alcohol gave a crystallin crop melting at 64° . It was recrystallized five times from ethyl acetate and melted at 65° . Lack of material prevented further purification. The analytical results indicate that the substance is a hydrocarbon possibly slightly impure hentriacontane (m. p. 68°).

Calculated for $C_{31}H_{64}$: C, 85.3; H, 14.7.

Found: C, 84.62; H, 14.76.

Concerning the material melting at 135° , recrystallization failed to alter the melting point. It was dried at 110° and analyzed.

Calculated for $C_{27}H_{46}O$: C, 83.9; H, 11.9.

Found: C, 83.72; H, 12.37.

The material is evidently a phytosterol. 1.0168 of anhydrous sub-

¹ See Power and Salway, *J. Chem. Soc.*, 97, 247 (1910).

stance, made up to 25 cc. with chloroform showed a rotation of -2.4° in a 2 dcm. tube, whence $[\alpha]_D^{20} = -29.5$. It yielded an acetyl derivative, which upon fractional crystallization showed a constant melting point, $122^\circ-123^\circ$.

The syrupy mother liquor remaining after these substances had been isolated was freed from alcohol and subjected to a fractional distillation and these fractions were again systematically redistilled. They were collected as follows: up to 190° ; $190^\circ-200^\circ$; $200^\circ-220^\circ$; $220^\circ-280^\circ$ and 280° up, the pressure being 16 mm. The first fraction was a liquid of a highly unsaturated nature.

0.1334 gram absorbed 0.1697 gram iodine; iodine number = 127.2.

Subst., 0.1153; CO_2 , 0.3448; H_2O , 0.1315.

Found: C, 81.55; H, 12.76.

The second fraction was an unsaturated solid.

0.1142 gram subst. absorbed 0.11225 gram iodine; iodine number = 98.3.

The upper fractions were extremely thick, sticky oils, which solidified upon cooling. They were not examined.

The Ether Extract of the Resin, which amounted to 51 grams was powdered and boiled with three liters of absolute ether. The mixture was filtered and the insoluble material washed with warm ether. This residue, which was a light green powder weighed 13.1 grams.

The material insoluble in ether was dissolved in 95% alcohol in which it was readily soluble, giving a black solution. It was subjected to prolonged boiling with animal charcoal in order to remove the coloring matter, but this effected very slight purification. The alcoholic solution was then concentrated slowly. At first several smeary products separated and after this a crystallin crop was deposited. Upon further concentration two more crystallin deposits separated and the mother liquor from the last gave an uncrystallizable syrup. The three crystallin deposits were separately boiled with small quantities of alcohol and this removed some material which was more readily soluble in alcohol than the main portion of these deposits proved to be. A small portion of each was then dissolved in chloroform to which a small quantity of acetic anhydride was added. When now a drop of sulfuric acid was added to each of these chloroformic solutions the first failed to show any color changes, but the last two showed a transient pink coloration passing to blue, and finally yielded a beautiful green color. These two were therefore united and crystallized from dilute pyridine, from which it separated in colorless microscopic curved needles that melted and decomposed at $285^\circ-288^\circ$. There was not enough for analysis, but it is evident that this substance belongs to that class of polyhydric alcohols of which ipuranol, so frequently found by Powers and his co-workers, is the best known example.

The substance soluble in ether, from which the above described 13.1

grams had been removed by filtration was concentrated and there separated out from a comparatively large dilution a slight crop of crystals which were filtered off and found to melt to an oil at 112°-114°. Further concentration did not increase the yield. The residue was therefore taken up in 500 cc. of ether and this solution was extracted with solutions of ammonium carbonate, sodium carbonate and potassium hydroxide and at length washed with water. Nothing was isolated from these alkaline extractions.

Upon concentrating the ether which had been extracted as described, another crystallin crop of the material melting at 112°-114° separated. It was joined to the material above and the total yield weighed 0.12 gram. Recrystallized once from alcohol the melting point remained constant.

Subst., 0.0788; CO₂, 0.2185; H₂O, 0.0891.

Found: C, 75.62; H, 12.65.

This neutral substance appears to be a new plant constituent, but since we did not have sufficient material to make a determination of the molecular weight no formula can be assigned to it.

The Chloroform, Ethylacetate and Alcohol Extracts of resin were examined but no crystallin substances could be isolated.

*Plant Ash Constituents.*¹—Portions of the ground and sifted leaves and tops, and some of the bulbs, respectively, were ignited below red heat in a muffle furnace. The charred material was extracted with hot water and filtered, the residue burned to a white ash and the filtrate mixed with this ash, and the whole evaporated to dryness on the water bath. The last portions of water were expelled by heating at 110°. The resulting

	Leaf.		Bulb.	
	I.	II.	III.	IV.
Moisture.....	3.79	..	2.04	..
Chlorine.....	0.30	..	0.19	..
Carbon dioxide.....	18.05	..	16.61	..
Sand.....	8.31	..	7.01	..
Carbon.....	0.71	..	0.48	..
Soluble silica.....	4.39	..	3.55	..
Sulfur trioxide.....	2.89	7.60 ²	3.33	9.42 ²
Phosphorus pentoxide.....	5.03	..	8.73	..
Ferric oxide.....	1.03	..	1.08	..
Aluminum oxide.....	2.55	..	1.08	..
Manganese.....	trace	..	trace	..
Calcium oxide.....	25.37	..	26.48	..
Magnesium oxide.....	5.34	..	5.02	..
Sodium oxide.....	5.58	..	4.68	..
Potassium oxide.....	20.64	..	20.35	..
Summation.....	102.98	..	100.63	..

¹ These analyses were conducted by Mr. H. H. Hill.

² These figures represent the total sulfur by the peroxide method and calculated as sulfate in terms of the ash.

solid was at once powdered and preserved in glass stoppered bottles until analyzed. The preceding table gives the constituents of the ash of the leaf and of the bulb.

LARAMIE, WYOMING.

A STUDY OF THE VELOCITY OF REACTION BETWEEN PHENOL AND FORMALDEHYDE.

BY BENJAMIN JABLONOWER.

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Introductory.—Of late, great interest is being shown in the products resulting from the condensation reaction between phenols and formaldehyde or other compounds containing the methylene group (CH_2). This reaction has been carefully studied by many, and there is an extensive literature on the subject. Very careful descriptions are given of the product at different stages of the reaction. For example, Dr. L. H. Baekeland has pointed out that three separate and distinct forms of this condensation product are possible, *viz.*, a liquid or a fusible solid, soluble in alcohol (A), an infusible solid which softens at higher temperatures (B), and finally a hard insoluble infusible solid (C).¹ Descriptions of these are very minute and all the details of procedure to obtain them are carefully set forth. But nowhere in the literature on this subject, at least as far as the writer is aware, is there any mention of any attempt to study this reaction from a physico-chemical point of view. The following is an account of an effort to obtain data of such a character.

While preparing some of this condensation product by the action of formaldehyde solution on carbolic acid in the presence of a very small amount of ammonia, with the aid of heat, the writer noticed that the liquid became more and more viscous under the influence of the heat applied. The usual method of following the course of this reaction is to observe the fluidity or stickiness of the liquid. It occurred to the writer that it perhaps would be possible to make a more careful study of the reaction by regular periodic measurements of the viscosity. This, it was thought could be taken as a means for the determination of the velocity of the reaction. But it was found that this method was impracticable. However, the viscosity of a liquid, among other things, is dependent on the density. The viscosity, n , of a liquid flowing through a tube is given by the well known equation.

$$n = \frac{\pi r^4 p t}{8 v l}, \text{ where}$$

r = the radius of the tube and

p = the pressure under which the volume, v , of liquid moves through the length, l , of the tube in the time, t .

¹ L. H. Baekeland, *J. Ind. Eng. Chem.*, 1, 149 (1909).