preserved juice will be the same as the original at the same temperature.

Preliminary experiments and one series of analyses indicate that mercuric nitrate may possess greater preserving action than mercuric potassium iodide.

ST. PAUL, MINN.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY.]

AN EXAMINATION OF THE LEAVES OF ADONIS VERNALIS.

BY FREDERICK W. HEYL, MERRISL C. HART AND JAMES M. SCHMIDT.

Received November 14, 1917.

Adonis vernalis L. is an anemonaceous plant of the family *Ranunculaceae*. It grows natively in Central Europe and in Asia, where it is known as the Bird's eye, Pheasant's eye or False Hellebore.

Extracts of the drug were first introduced into medicine in 1879 by Bubnow,[1] who employed them as a cardiac stimulant. Cervello,[2] interested by the favorable report of Bubnow, described a method for the preparation of an active digitalis like glucoside from *Adonis vernalis*, following the method employed in the manufacture of "Digitalin German" from Digitalis seeds. The plant was macerated for two days with 50% alcohol, and the extract after filtration was precipitated with lead subacetate. The concentrated filtrate was rendered ammoniacal and the glucoside was precipitated with tannic acid. The precipitate was washed with water and boiled with alcohol in presence of zinc oxide. After evaporation of the alcohol the residue was extracted with absolute alcohol and coloring matter was precipitated from the concentrated solution by the addition of ether. The filtrate from this contained his active glucoside principle which he named adonidin. The chemical work is entirely indefinite, but the pharmacology of this preparation was well worked up. Cervello found that "adonidin" was ten times as active as a preparation Digitoxin(?) used by Koppe[3] in bringing about heart rest in frogs. In contrast to this we find that dosage of adonidin (Merck) given as high as 0.03 g. which is about fifteen times the dose for the digitoxin and about equal to that given for the mixed glucosides from digitalis seeds.

Kramer[4] worked up a parcel of *Adonis aestivalis* by extracting with 96% alcohol and adding an equal volume of water to the extract. This mixture was shaken with petroleum ether, ether, and with chloroform. The chloroform extract yielded an active glucosidic principle which could be precipitated from concentrated alcoholic solutions by the addition of

[1] "Dissertation," St. Petersburg (1880).

[2] *Arch. exp. Path. Pharm.*, **15**, 235 (1882).

[3] R. Koppe, *Ibid.*, **3** (1879).

[4] *Arch. Pharm.*, **234**, 452 (1896).

ether. He ascribed to his preparation the formula $C_{25}H_{40}O_{10}$, and to this is also assigned the name adonidin according to Abderhalden's Handlexikon,[1] but this is incorrect as there is a marked difference in the action of this principle with the one obtained from *Adonis vernalis*. Thus when a preparation of adonidin prepared from the latter plant by Merck was compared by Kobert with Kromer's preparation it was found that 0.04 mg. of the preparation by Merck, subcutaneously injected into the frog, brought about the typical heart rest and killed the frog in $^3/_4$ hr., while 200 times this quantity of Kromer's preparation failed to bring this about.

Mordagne[2] stated that he obtained only two grams of adonidin from 10 kg. of *Adonis vernalis*. This amounts to only 0.02% of the drug. He reported the results of his analyses as showing, C = 42.6%; H = 7.75%.

More recently several further reports on pharmacological studies have been reported by Fuckelman,[3] Slovtzov,[4] Chevalier,[5] and Mercier,[6] Although it has been recently stated that crude adonidin has been shown to be a mixture, nothing definite has been isolated. These recent papers also disagree among themselves as to the proper method for classifying the drug, some comparing adonis with digitalis, others with squill. References may be found in the medical literature recording the use of this drug in cardiac dropsies and in epilepsy. In the form of the common fluid extract *Adonis* is included in the new National Formulary, where the average dose is given as 0.125 mil or about 2 minims.

Our attention was directed to this plant when, in the process of manufacturing the tincture, a standard seemed desirable. Recent work by Hatcher and Brody,[7] using the intravenous cat method for purposes of determining relative toxicity, showed that the relation between adonidin and ouabain approximated 30:1, respectively. Two lots of drugs, one of which cost four times as much as the other, were made up into tinctures by cold percolation (10 g. per 100 cc.). These were assayed by the one-hour frog method[8] which is official for tinctures of digitalis, strophanthus, and squill. The hearts stopped in systole in a manner similar to that observed when poisoned with digitalis.

From this table it is seen that the average toxicity of tinctures made with more dilute alcohol is somewhat greater than when 95% alcohol is used; also the tinctures prepared with 95% alcohol proved to be more

[1] *Biochem. Handlex.*, **2**, 639 (1911).
[2] *Pharm. J. Trans.*, **16**, 145 (1885).
[3] *Chem. Zentr.*, 1, 430 (1912).
[4] *Russki Vratch*, **2**, 1 (1913).
[5] *Zentr. Biochem. Biophys.*, **15**, 208.
[6] *Ibid.*, **16**, 652.
[7] *Am. J. Pharm.*, **82**, 360 (1910).
[8] *U. S. Pharmacopoeia*, **9**, 606 (1916).

stable when examined in this manner. The average M. S. D.[1] of a tincture of *Adonis vernalis* (95% alcohol) we may put at 0.0045 cc. per g. frog. In all these assays the varying resistance of the frogs was taken into consideration, as the M. S. D. for each lot was determined, using crystalline ouabain.

Sample.	Menstruum. % alcohol.	1st assay. Mil.	Time elapsing. Days.	2d assay. Mil.	Loss %.
A.....	50	0.0027	205	0.0039	31
A.....	60	0.004	205	0.005	20
A.....	70	0.0033	205	0.0039	16
A.....	80	0.0033	210	0.0039	16
A.....	95	0.0043	315	0.005	14
A.....	95	0.0043	...	..	..
B.....	50	0.0032	...	..	..
B.....	50	0.0043	...	..	..
B.....	95	0.0055	196	0.006	9
B.....	95	0.0045	...	..	..

Adonis vernalis is therefore more potent than digitalis in terms of this assay process.

Ouabain = 0.0_{65} g. per g. frog.
Digitalis leaves = 0.0_{36} g. per g. frog.
Adonis vernalis leaves = 0.0_{345} g. per g. frog.
Strophanthus = 0.0_{56} g. per g. frog.

It would therefore appear reasonable to adopt the same standard for both these drugs; *i. e.*, 0.006 cc. of the tincture per g. frog.

Turning our attention to the active principle adonidin, we examined two samples procured from Merck. The first, which had been obtained in a sealed tube a number of years ago, appeared to be the product of Cervello's process. It had a dark, molasses-like consistency, and was amorphous and hygroscopic. It is stated in the Index (1902) to be prepared from the herb (kraute). The more recent sample was a brownish yellow amorphous, hygroscopic powder. Merck's new Index (1907) states that it is prepared from the root of *Adonis vernalis* L. and other species of *Adonis*. The material is a mixture melting very indefinitely, softening at 97°, but did not run down at 133°, where decomposition began.

Tests on Merck's Adonidin.—For glucosidic character: 0.4024 g. was dissolved in 50 cc. water. Of this solution 25 cc. gave no reduction directly with Fehling's solution. The other half was heated with 5.5 cc. 38% hydrochloric acid for one hour. The solution was neutralized. Examined by the Walker-Munson process, 0.1066 g. Cu_2O equivalent to 0.0462 g. glucose was obtained. Thus 22.9% glucose (?) was liberated.

The M. S. D. determined by the one-hour frog method was 0.0_{55} g. per g. frog.

These values obtained by pharmacological assaying indicate the presence of approximately 1% of an active constituent, assuming, of course, that the adonidin of Merck is present in the leaf.

[1] Minimum quantity dose producing permanent systole of the frog's ventricle at the end of exactly one hour.

Our further work in the endeavor to isolate a toxic glucoside proved that the presence of a glucoside in the leaf is questionable. We have shown that a very considerable proportion (over 60%) of the toxicity can be accounted for in the basic fraction precipitated with phosphotungstic acid. Even by acid hydrolysis of fractions of the various plant extracts (which should contain a glucoside if such were present) we obtained but the slightest evidence of the presence of "adonidin." It appears to us that the tannic acid precipitate of Cervello, Mordagne and others who worked with the leaves, were amorphous, hygroscopic mixtures, containing basic substances. While the use of tannic acid as a precipitant for glucosides has been of advantage in many instances; *e. g.*, strophanthin, antiarin, there will also be found described in the literature as glucosides a number of substances precipitated from plant extracts with tannic acid, which are doubtless mixtures.

Our sample consisted of the herbaceous parts in the flowering state and was of German origin (Leipsig).

The proximate analysis shows that the air-dried leaves contained 8.14% moisture, and 10.2% ash. Ligroin extracted 1.78%; ether, 2.5%; and alcohol, 18.6%. The residue insoluble in alcohol had the following composition: crude fiber, 18.65%; pentosans, 14.2%; protein, 14.1%; starch, 1.1%; dextrin, 0.5%. The resin amounts to 3.37%.

The products present in the alcoholic extract and soluble in water yielded, when shaken with ether, a neutral substance melting at 133° and agreeing in composition with formula $C_{14}H_{18}O_4$. The aqueous solution yielded a heavy lead subacetate precipitate. When this was decomposed we were able to prepare from it a yellow complex acidic substance agreeing with the formula $C_{31}H_{32}O_{16}$. It did not melt when heated to 285°, browning at 240°. It formed a beautifully crystalline decacetyl derivative $C_{31}H_{22}O_6(C_2H_3O_2)_{10}$, melting sharply at 190–192°. The filtrate from this lead salt yielded 1.2% levulose, 2.9% adonitol,[1] and lastly choline, $C_5H_{15}O_2N$.

The ligroin extract of the resin yielded a quantity of phytosterolin, and a mixture of a hydrocarbon and of a higher alcohol which had the melting point of myricyl alcohol, 81–83°. The phytosterol fraction consisted chiefly, but not entirely, of a sterol melting at 138–9°, and possessing the formula $C_{27}H_{46}O$. The corresponding acetate melts at 127–128°. The fatty acids were made up to a very large extent of palmitic and linolic acids.

The ether extract of the resin yielded in the usual manner a pure sample of phytosterolin, $C_{33}H_{56}O_6$, melting at 285–295° and forming an acetate which melted at 167–168°.

The chloroform extract of the resin yielded a further quantity of phytosterolin.

[1] Merck, *Archiv. Pharm.*, **231**, 127 (1892).

The ethyl acetate and alcoholic extracts of the resin yielded little of interest, a small quantity of adonitol being isolated, which had apparently adhered to the resin.

The presence of aconitic acid, which was reported by Linderos,[1] we were unable to confirm.

EXPERIMENTAL.

A. Proximate Analysis.

Quantitative extractions of the air-dried leaves with various solvents gave the following results:

Extract.	Per cent.
Ligroin (35–55°)	1.78
Ether (110°)	2.49
Alcoholic	18.59

The proximate analysis gave the results tabulated below:

	Per cent.		
Moisture	8.14	Protein	14.1
Starch (diastase)	1.1	Ash	10.2
Pentosans	14.2, 13.95[a]	Dextrin	0.5
Crude fiber	18.65		

[a] On residue after alcohol extraction.

Quantitative estimations on the alcohol soluble carbohydrates resulted as follows:

A large quantity of the drug was exhausted by cold percolation with 50% alcohol. The percolate was concentrated to a comparatively small volume, and precipitated with an excess of lead subacetate solution. The excess of lead was precipitated with sodium phosphate, and then the solution was made up to a definite volume (1500 cc. = 1080 g. drug). 25 cc. of the solution (18 g. drug) were diluted to 50 cc. The reading in a 1 dcm. tube was —1.4° at 23°. Gravimetric estimations upon this solution by the Walker-Munson process gave, respectively, 0.05 and 0.0515 g. Cu_2O with 5 cc. aliquots. Levulose = 1.2%.

When 25 cc. of the above solution were acidified with 2.5 cc. of 40% hydrochloric acid, permitted to stand overnight and then neutralized and made up to a volume of 50 cc., the rotation in a 1 dcm. tube was —0.5°. Gravimetric estimations with Walker-Munson process failed to show any increase in reducing sugars. Sucrose is absent. This is indicative of considerable stability on the part of the glucoside.

The leaves were examined for the presence of alkaloids according to the method which is official for belladonna, but the results were negative.

B. The Solubilities of the Toxic Constituent and the Presence of a Glucoside.

It was shown by Barger and Shaw[2] that in the case of tincture of digitalis a very considerable part of the toxic constituents adhere to the resin which is precipitated by the addition of water. Consequently they are

[1] *Ann. Chem.*, **182**, 365 (1876).

[2] *Pharm. J.*, **73**, 249 (1904).

not found quantitatively among the water soluble constituents. Thus, in one case, they found $^{25}/_{29}$ of the toxicity in the resin.

This difficulty might be emphasized in the case of *Adonis vernalis* because of the large quantity of resin present. In the following experiments it will be observed that the toxicity resided quantitatively in the water-soluble constituents even after clarifying with lead subacetate.

Attempt to Prepare "Adonidin" by Using Ethyl acetate.—A quantity of the leaves of *Adonis vernalis* was completely extracted with 50% alcohol, and the percolate concentrated to such a volume that 500 cc. was equivalent to 1080 g. of the drug.

One liter of this solution (2.16 k.) was directly precipitated with basic lead acetate solution[1] (1050 cc.). The filtrate and the washings from the lead precipitate was divided into two equal parts, A and B.

A. This solution was freed from lead by the use of Na_2HPO_4; and then the last trace remaining in the filtrate was removed with hydrogen sulfide and filtering. Eight grams of calcium carbonate were added and the solution was concentrated to a volume of 1500 cc. Twenty-five cc. were removed for the estimation of carbohydrates as described above.

The remainder of Solution A was evaporated to dryness at 45–50° and taken up in 2 liters absolute alcohol in which it did not entirely dissolve. The solution was halved at this point, each part (1 liter) being equivalent to 531 g. *Adonis vernalis*.

Pharmacological examination, by the one-hour frog heart process was made upon this solution.

Ten cc. (5.3 g.) diluted to 50 cc., gives a solution 1.06 × tincture strength. The quantity required to stop the frog heart in one hour, per 1 g. frog, was 0.00292 cc. or calculated as tincture, 0.0031 cc.

This extract contained 21.1% of the drug in solution, *i. e.*, 112 g. in 1000 cc.

The toxicity of this material therefore 0.000065 g. per 1 g. frog. The solution contained considerable ash (5.6% drug).

The remainder of this aliquot (518 g. drug) was concentrated to 250 cc. and precipitated with ordinary ethyl acetate. The supernatant fluid was decanted. The precipitate was redissolved in absolute alcohol and reprecipitated with absolute ethyl acetate. The alcoholic-ethyl acetate filtrates were united, concentrated and again treated with absolute ethyl acetate (500 cc.). The liquid was decanted from a small amount of syrup and the process was repeated. Eventually an ethyl acetate solution was obtained which retained 28% of the original toxicity of the drug, the remainder having been lost in the syrupy precipitates insoluble in ethyl acetate. The dissolved solids showed a toxicity of 0.0000056 g. per 1 g. frog.

The material weighed 2.6 g. but it was only very slightly glucosidic,

[1] U. S. Dept. Agr., Bur. Chem., *Bull.* 107 (revised), 40.

and furthermore, it contained a high proportion of nitrogenous substances. The solution had a volume of 50 cc.

5 cc. (0.2615 g.) gave 0.0029 g. Cu_2O. 5 cc. heated on a boiling water bath for one hour with 50 cc. of approximately 7% hydrochloric acid gave 0.0180 g. Cu_2O. Hence approximately 2.3% glucose was liberated.

5 cc. required by the Kjeldahl method 17.05 cc. 0.05 *N* acid equivalent to 4.6% nitrogen.

Preparation of the "Adonidin" Fraction Using Hydrogen Sulfide to Remove Pb" Instead of Na_2HPO_4.—1.08 kg. drug were percolated to exhaustion with 7.6 liters of 50% alcohol. The green percolate was concentrated to a volume of 500 cc., and precipitated with 1050 cc. lead subacetate solution. After standing overnight the voluminous yellow precipitate was filtered off and washed three times by trituration with water (500 cc., 250, 250). The brilliant yellow filtrate (3.7 liters) was made up to a volume of 5 liters with 95% alcohol and divided in to five aliquots, each of which was saturated with hydrogen sulfide ($^1/_2$ hour), immediately filtered free from precipitated lead sulfide, and at once concentrated. Each aliquot contained 7.56 g. acetic acid at the beginning of the concentration and after concentrating (at 40°) to a volume of 630 cc. 6.88 g. remained.

In order to determine the reducing action on Fehling's solution before concentration, an aliquot was analyzed.

A quantity (equivalent to 1.8 g. *Adonis vernalis* gave 0.0655 g. Cu_2O = 0.0364 g. Cu_2O per 1.0 g. = 1.56% glucose.

The solutions were now concentrated to a syrup and taken up in 95% alcohol (2.5 liters). There ensued a viscous syrupy separation amounting to 30 g. (2.8% of the drug) which consisted in part of calcium sulfate.

The 95% alcoholic solution was analyzed as follows:

Acetic Acid: 8.0 g.; *direct reducing action* with Fehling's: 0.0331 g. Cu_2O per 1.0 g. plant; *reducing action after heating for 1 hr.* with 10% dilute alcoholic hydrochloric 0.0441 g. Cu_2O per g. plant. *Total solids* dried at 100° = 16.8%; dried at 110° = 16.03%. *Ash* = 3.08%. *Toxicity of solution of tincture concentration* = 0.00040 cc. per g. frog.

This 95% alcoholic solution equivalent to 1047 g. solution was concentrated to a syrup and taken up in about 2.5 liters absolute alcohol. A second heavy viscid syrupy mass separated. After standing overnight the clear supernatant alcoholic solution was decanted from the solid (23.2 g.) and concentrated to a syrup, taken up in about 300 cc. absolute alcohol, a little ethyl acetate was added and the solution was put aside in the ice chest. No crystals separated after standing several weeks.

It was concentrated to a syrup and again taken up in absolute alcohol and made up to a volume of 2000 cc. A precipitate (15 g.) separated. The solution at this point should, it would seem, contain the glucoside, and

the repeated treatment with absolute alcohol should have removed the sugars and other impurities and we should now have a purified preparation of the glucoside (plus the adonitol). We were unable to separate adonitol by direct crystallization.

An examination of the toxicity indicated that 50% of the toxicity had been lost perhaps through the precipitation of the 30.3 + 23.2 + 15 g. of material above mentioned.

The solution (1047 g. drug) contained 120 g. soluble material (11.4%). of which 21.8 g. was ash. 5 cc. (0.3003 g.) diluted to 26.17 cc. showed a toxicity of 0.0079 cc. per 1 g. frog. Toxicity of residue = 0.000092 g. per 1 g. frog, making it actually weaker than before.

Furthermore, this solution, which should contain the glucoside, gives little or no evidence of the presence of a glucoside when examined by the Walker-Munson process.

(1) Direct reduction: 15 cc. gave 0.1144 g. Cu_2O.

(2) Invert reduction: 15 cc. + 10% hydrochloric acid in dilute alcohol heated for one hour on the water bath gave 0.1246 g. Cu_2O. By calculation the inversion has produced 0.0102 g. Cu_2O more equivalent to 0.004 g. glucose. Since 15 cc. represents 7.85 g. plant this represents approximately 0.05% glucose which at most would represent 0.15% of a glucoside.

The acidification of the solution in presence of absolute alcohol yielded a heavy precipitate which gave a positive test for potassium chloride and it also contained. traces of sodium, calcium and magnesium.

C. Complete Examination of Alcoholic Extract.

For this purpose 34 k. were exhausted by percolation with cold wood alcohol. The percolate (318 liters) was concentrated under diminished pressure to a volume of 7.85 liters.

This concentrated extract was poured into 16 liters of distilled water and vigorous shaken. A heavy, green, fatty resin separated. After standing overnight the aqueous layer was decanted through a wetted filter paper and the resin was washed with fresh additions of distilled water.

The Examination of the Water-Soluble Constituents.—This solution (22 liters) containing the water-soluble constituents of 34 kg. of *Adonis vernalis* was concentrated under diminished pressure to a volume of 6.25 liters. The concentrated extract was extracted repeatedly with large volumes of ether. The ether was removed from these extractions and the residues found to be small in amount. They were redissolved in absolute ether, and the ethereal solution was washed with water and extracted in the usual manner with solutions of ammonium carbonate, sodium carbonate and of potassium hydroxide, but the amounts were slight and smeary. The ethereal solution after these extractions was allowed to evaporate spontaneously, whereupon a gradual accumulation of colorless plates separated. This product, upon recrystallization from

alcohol, melted sharply at 133° to a clear oil. This neutral substance amounted to 0.45 g. and agreed in composition with the formula $C_{14}H_{18}O_4$.

0.1317 g. subst. gave 0.0825 g. H_2O and 0.3243 g. CO_2. Found: C, 67.2; H, 7.0. Calc. for $C_{14}H_{18}O_4$: C, 67.2; H, 7.2.

The aqueous solution which had been extracted with ether was now repeatedly shaken with chloroform, which removed 55 g. of indefinite material. The aqueous layer at this point measured 6.8 liters. The distribution of nitrogen in this solution is as follows:

10 cc. required 36.9 cc. of 0.1 *N* acid, equivalent to 0.05166 g. nitrogen.

20 cc. gave, with lead subacetate, a precipitate containing 0.0182 g. nitrogen.

25 cc. gave, when distilled with MgO, 0.04004 g. nitrogen as ammonia.

The distribution of nitrogen is therefore as follows: Total soluble nitrogen = 0.103%; ammonia nitrogen = 0.032%; lead subacetate precipitable nitrogen = 0.018%; nitrogen precipitated with phosphotungstic acid = 0.056%.

A part of the solution (500 cc.) failed to yield any appreciable precipitate with mercuric acetate when separately examined for asparagine, glutamine and allantoine.

An aliquot of the above-mentioned solution, equivalent to 15 kg. of the plant, was extracted with hot amyl alcohol. The material extracted amounted to approximately 50 g. and failed to yield anything of interest upon further investigation.

The aqueous solution was now diluted to a volume of about 10 liters and precipitated with an excess of basic lead acetate. After standing 24 hours, the bright yellow precipitate was filtered off on suction and washed by agitation with several liters of distilled water and again filtering. The precipitate was suspended in about 20 liters of water and decomposed by saturation with hydrogen sulfide. The filtrate from the lead sulfide was concentrated to a volume of about 500 cc. and permitted to stand for several months. Ten grams of a canary yellow substance separated and was filtered off on suction. This material was treated with boiling water and after filtration treated with boiling 95% alcohol. It was dissolved in pyridine and fractionally precipitated by two additions of anhydrous ether. The first fraction precipitated as an oil but solidified to a crystalline deposit on standing. It had a slightly dirty yellow color. It was dissolved in pyridine, and alcohol was added and the brownish yellow colored crystals which deposited were digested with ether, dried at 150–160° and analyzed (C = 57.0; H = 4.58). The second fraction was a pure lemon-colored precipitate that formed plates on standing. These crystals contained pyridine of crystallization. After digestion with ether and subsequent filtration they were dried at 150–160° and analyzed.

I. 0.1787 g. subst. gave 0.3713 g. CO_2 and 0.0722 g. H_2O, C = 56.66; H = 4.53.

II. 0.1113 g. subst. gave 0.2310 g. CO_2 and 0.043 g. H_2O, C = 56.6; H = 4.34.

III. 0.5296 g. crystals dried *in vacuo*, lost 0.0805 g. pyridine at 150–160°, C_5H_5N = 15.2%.

Calc. for $C_{31}H_{30}O_{16}$: C = 56.5; H = 4.6.
Calc. for $C_{31}H_{30}O_{16}$: C = 56.3; H = 4.8.
Calc. for $C_{15}H_{16}O_8$: C = 55.5; H = 4.9.
Calc. for $C_{31}H_{32}O_{16} + 1.5C_5H_5N$: C_5H_5N = 15.2%.

The acid decomposed indefinitely, depending on the rate of heating. The pyridine solution, diluted by the addition of water, gave a dark olive-green color with a solution of ferric chloride; with normal lead acetate a yellow lead salt precipitated voluminously. Determination of methoxy group by the Perkin process proved negative.

In the meantime the main filtrate from which the above described yellow substance had separated gradually deposited during several months 7.9 g. further of this substance (C = 56.5; H = 4.8). This product was acetylated, by dissolving in a minimum quantity of pyridine, adding an excess of acetic anhydride and boiling on a reflux for two hours. The crude acetate is precipitated upon the addition of water. It crystallizes from alcohol in rosets of radiating needles, but many crystallizations are required to raise the melting point from 185–190° to a sharp melting point at 191–2°. Acetylization, using acetic anhydride and sodium acetate, did not proceed as smoothly.

The determination of the acetyl radicals was made by Perkin's[1] process.

0.2 g. required 18.5 cc. 0.1 *N* alkali to neutralize acetic acid. Found: 54.6%. (The yellow substance was recovered.)

0.1007 g. gave 0.2094 g. CO_2 and 0.0457 g. H_2O. Found: C = 56.7; H = 5.08.

Calc. for $C_{51}H_{50}O_{26}$: C = 56.75; H = 4.7; $10(C_2H_3O_2)$ = 54.7; $C_{51}H_{52}O_{26}$: C = 56.7; H = 4.9; $10(C_2H_3O_2)$ = 54.6.

The acetate is a deca-acetate, $C_{31}H_{22}O_6(OCOCH_3)_{10}$. The material is not glucosidic. It is insoluble in cold aqueous alkali. An alkaline fusion revealed nothing of interest.

The filtrate from this yellow substance (containing 296 g.) did not contain tannic acid. The solution was made up to 570 cc. and of this 150 cc. was diluted to 300 cc. and enough sulfuric acid was added to make the concentration 5%. Upon boiling for several hours 1.5 g. of the yellow substance separated. Further boiling yielded only resin and some calcium sulfate. The aqueous liquors yielded nothing of interest, except a trace of an osazone. An alkaline hydrolysis with 10% potassium hydroxide for two minutes resulted negatively.

The yellow substance is an alteration product, but not of a glucoside:

Some of the yellow lead salt was washed repeatedly with large volumes of water until the washings were free from sugar. The precipitate was then suspended in 6 l. water and decomposed with hydrogen sulfide. The lead sulfide was filtered off, the

[1] *J. Chem. Soc.*, **87**, 107 (1905). We repeated this on a 1.0 g. sample using 0.5 *N* solutions. There was required 19.2 cc. 0.5 *N* alkali. Found: $(C_2H_3O_2)$ = 56.6%. $C_{31}H_{21}O_5(C_2H_3O_2)_{11}$: requires 57.8%.

filtrate was concentrated, and hydrolyzed with 5% H_2SO_4. The sulfuric acid was removed quantitatively. By the Walker-Munson process it was estimated that this solution should contain 10.1 g. sugar, it being strongly reducing. This reduction is not due primarily to glucose, because upon examining this syrup it was possible to isolate but a trace of *d*-phenylglucosazone decomposing at 207–208°.

The filtrate from the basic lead acetate precipitate was freed from the excess of lead with hydrogen sulfide, and after filtering off the lead sulfide the filtrate was concentrated to a syrup that measured 1200 cc. Nothing could be separated from this solution in the crystalline condition.

A portion (25 cc.) was examined for sugars. With phenyl hydrazine a crystalline deposit amounting to about one gram of *d*-phenylglucosazone was obtained. The melting point upon recrystallization was 210°. Pentose sugars could not be detected.

Another portion (545 cc.) equivalent to 6.8 kg. of the drug was precipitated with an excess of phosphotungstic acid in the presence of 5% sulfuric acid, and a heavy precipitate resulted. This was removed by filtration and washed with 5% phosphotungstic acid wash and decomposed by the method of Wechsler. The solution of the bases was concentrated to a small volume and digested with absolute alcohol. A considerable quantity of insoluble material was removed by filtration and the filtrate was again evaporated to dryness and treated as before. The absolute alcoholic solution was acidified with hydrochloric acid, but after prolonged standing no crystals separated and the solution had become concentrated to the consistency of a syrup. This residue was fractionally extracted with absolute alcohol.

The first extract of most readily soluble substances was fractionally precipitated with a saturated alcoholic solution of mercuric chloride and the 1st and 3d fractions were crystalline, while the intermediate fraction was smeary.

The first fraction of the crystalline mercuric chloride was dissolved in water and the solution was filtered in order to remove a small amount of brown amorphous material This solution yielded three successive crops of prismatic crystals, which were decomposed with hydrogen sulfide. The filtrate from the mercuric sulfide was allowed to evaporate to dryness over lime in a desiccator. The crystalline residue consisted in each case of needles, which were very soluble in water and in alcohol. They were identified as choline chloride by the analysis of the chloroplatinate that melted upon rapid heating at about 236–238°.

Calc. for $(C_5H_{14}ON)_2PtCl_6$: Pt 31.66. Found: I, Pt, 31.15; III, 31.4.

The third crystalline precipitate of mercuric chloride above mentioned was likewise shown to yield choline chloroplatinate. (Found: Pt, 31.3.) The total yield of choline amounts to about 1.7 g., or approximately 0.025% of the drug.

A third portion of the syrupy filtrate from the basic lead acetate precipitate (freed from lead) amounting to 7.9 kg. of the drug, was sharply concentrated and then taken up as completely as possible with boiling 95% alcohol. The solution was again evaporated to dryness under diminished pressure in order to remove any water. The residue was boiled with two liters of absolute alcohol and, after standing, the clear, hot supernatant liquid was decanted from the syrup which proved insoluble. The syrup was treated in the same manner with three further portions of absolute alcohol.

From these alcoholic solutions there separated a quantity (about 190 g.) of a crystalline adonitol, which was previously reported by E. Merck.[1] It was shown by E. Fischer[2] to be the pentahydric alcohol obtained by the reduction of ribose. The melting point without crystallization was 98–102°. Our yield was 2.4%. Merck reported about 4.0%.

These alcoholic solutions, after bringing the adonitol to separate as completely as possible, were examined for glucosides. The first extract was made quantitatively to a volume of 300 cc. Its examination pharmacologically by the one-hour frog method, indicated that the preceding extensive operations had deprived this solution of its activity.

A solution made so that it represented fluid extract strength gave: M. S. D. = 0.0086 cc. and 0.0085 cc., whereas an active fluid extract should give M. S. D. = 0.00045 cc. The fraction retains only 5% of the activity.

The second alcoholic extract was still less toxic, and the third was inactive.

The first fraction was slightly acidic. The syrup when dissolved in water yielded an abundant precipitate, when ammonia and tannic acid were cautiously added, but upon treating this precipitate with freshly prepared lead oxide in the usual manner, no "adonidin" resulted, the lead apparently combining with the acidic product under examination.

The syrup, insoluble in absolute alcohol, yielded nothing crystalline. When distilled with hydrochloric acid it could be shown to yield a small quantity of furfural, as phloroglucid could be precipitated in the distillate.

Toxicity of the Base Fraction.—The substances precipitated with phosphotungstic acid were re-examined quantitatively in order to see if we could in this fraction account for any part of the characteristic action of the drug on the frog heart. This work indicated that over 60% of the toxicity is found among the basic substances.

Solution B (see page 441) representing 1080 g. of the drug was evaporated to dryness, taken up in alcohol. A heavy precipitate was separated and the alcoholic filtrate was concentrated at 40–45° to a syrup. It was again taken up in absolute alcohol and another precipitate was filtered off. The solution was concentrated and an unsuccessful

[1] *Loc. cit.*

[2] *Ber.*, **26**, 633 (1893).

effort was made to quantitatively separate adonitol by prolonged standing in the ice chest. The solution was made to a volume of 2 l.

Five cc. were diluted to 20 cc. (1.35 × tincture). This gave M. S. D. = 0.0059 cc. per g. frog. Or calculated to tincture strength M. S. D. = 0.00797 cc. The alcohol was removed, the syrup taken up in 5% sulfuric acid, and the bases were precipitated with phosphotungstic acid in the usual manner. The bases were liberated with baryta and the solution thereof made up quantitatively to a volume of 500 cc. (1075 g. drug).

This solution gave M. S. D. = 0.0006[1] cc. per g. frog. Calculated to tincture strength we have M. S. D. = 0.013 cc. per g. frog; *i. e.*, 61% of the toxicity of the solution resides in this fraction.

25 cc. (53.75 g. drug) required 21.4 cc. 0.1 *N* acid by the Kjeldahl process, equivalent to 0.03 g. *N* or 0.056%.

This fraction contained 14.4 g. of solids (dried at 100°). The ash amounted to 3.24 g. The fraction yielded a precipitate with tannic acid which was soluble in ammonia.

Several other efforts were made to prepare fractions of the water-soluble constituents which might contain the toxic substance in higher concentration. We endeavored to extract it from the aqueous liquor after defecating with lead subacetate solution. This process was used by Tahara[2] to separate adonin from *adonis amurensis*. An aqueous solution, defecated with lead subacetate solution and then treated with sodium sulfate and containing the extractives from 7.47 kg. drug in a volume of 1.9 liters was repeatedly extracted with large volumes of chloroform (12 liters). The chloroform was dried by shaking with sodium sulfate, concentrated to a syrup in the presence of some calcium carbonate, and then taken up in absolute alcohol. The extracted matter, which had a very unpromising appearance, weighed 5.4 g. When dissolved in alcohol and diluted so that the solution represented the concentration of a tincture it was found that M. S. D. = 0.016 cc. per g. frog. This material reduced Fehling's solution directly. Heating with acids increased its reducing power. This material is exceedingly active in producing the stoppage of the frog heart. ($0.0_5 12$ g. per g. frog = M. S. D.) After standing for months, drying over sulfuric acid, the activity remained practically unchanged. This substance was not precipitated with tannic acid but gave a heavy test with phosphotungstic acid.

The process of salting out with ammonium sulfate, which Thoms[3] used to separate strophanthin, was unsuccessfully attempted.

Cervello's work was repeated and an amorphous product, somewhat more toxic than commercial "adonidin," resulted. When hydrolyzed with dilute hydrochloric acid it did not appear to be entirely glucosidic. However, the material failed to give a test with phosphotungstic acid and nitrogen was present to the extent of only 0.3%.

[1] Contains 0.000017 g.

[2] *Ber.*, **24**, 2581 (1891).

[3] *Ibid.*, **31**, 271 (1898).

The ground leaves (2 kg.) were exhausted by percolation with 14 liters of 50% alcohol. This percolate was concentrated to a volume of 6 liters and 2.16 liters of lead subacetate solution was added. The yellow lead salt was filtered out on a Büchner, washed with water and to the filtrate 240 g. of sodium phosphate was added. The filtrate and washings from the lead phosphate measured 8730 cc. The solution examined pharmacologically showed a toxicity of 0.0057 cc. per g. frog when diluted to tincture strength.

This solution was concentrated at 50° to a volume of approximately 750 cc., 1.5 cc. of 1% ammonia water was added and a complete precipitation effected with 19.6 cc. of 20% tannic acid solution. The light precipitate was filtered off, washed with water, and triturated with freshly precipitated zinc oxide. The mixture was dried in a vacuum and then digested with cold 95% alcohol. The alcoholic filtrate and washings was concentrated to a syrup and made to a volume of 25 cc. with absolute alcohol.

The solution contained 2.43 g. Toxicity = 0.00003 cc. per g. frog. This represents 24% of the total toxicity of the drug. This volume of solution contains $0.0_{6}29$ g. Cervello's "adonidin."

0.559 g. required by the Kjeldahl process 1.2 cc. 0.1 *N* acid = 0.3%.

0.224 g. gave with the Munson-Walker process 0.0042 g. Cu_2O, equivalent to 0.79% sugar calculated as glucose(?).

0.224 g. heated with dilute alcoholic hydrochloric acid (10%) gave on reduction 0.0438 g. Cu_2O. The sugar split off calculated as glucose amounts to 0.0167 g. or 7.5%.

The residue from the cold 95% alcoholic extract above described was extracted with boiling 95% alcohol and 1.388 g. further was obtained. The material was similar to that obtained by cold extraction. The material showed a toxicity of $0.0_{5}37$ g. per g. frog.

The Examination of the Resin.—The resin which precipitated when the alcoholic extract was poured into water weighed about 1146 g., equivalent to 3.37% of the drug. This resin was redissolved in alcohol and then poured upon purified sawdust, dried, and transferred to a continuous extractor and extracted with the following results:

Petroleum ether (40–60°)	740 g.
Ether	124
Chloroform	85
Ethyl acetate	14
Alcohol	90
Total	1053

The Ligroin Extract.—One-half (370 g.) of this fraction was dissolved in ether and shaken with solutions of potassium hydroxide (5% and 10%). The alkaline solutions were acidified and extracted with ether. On extracting the ethereal solution with ammonium carbonate an emulsion formed. The solution was filtered and a small quantity of a black gummy solid was obtained. This was washed with dilute alcohol and the residue crystallized from dilute pyridine. The resulting crystals were well washed with alcohol and ether and again crystallized from dilute pyridine. It

gave the characteristic color tests for phytosterolin. It melted at 275–285°. It weighed about 0.43 g.

The material soluble in ammonium carbonate consisted of 20 g. of a blackish green resin from which nothing was isolated.

The ethereal solution was now extracted with solutions of potassium carbonate, and the fatty acids occurring free in the plant were removed. The alkaline extract containing the potassium salts of these fatty acids was acidified and extracted with ether. The ether was removed and some resin was removed by dissolving the residue in petrolic ether. The petrolic ether was dried over anhydrous sodium sulfate. The ether was removed and a residue of about 130 g. was obtained. This was distilled under diminished pressure. The iodine number of the distilled acids which solidified in the receiving tube was found to be 94.4. A very considerable quantity of this material (33 g.) could not be distilled and it remained as a tar in the flask. These fatty acids were studied in connection with those obtained upon the subsequent hydrolysis of the glycerides.

The ether solution which had been extracted with ammonium carbonate and potassium carbonate was now extracted with a solution of potassium hydroxide. On acidification a slight amount of tarry material was obtained from which nothing of a crystalline nature was isolated.

The original ethereal solution of the fat which had been extracted with solutions of potassium hydroxide was evaporated to dryness and the residue, which weighed 177 g., was saponified by boiling with 10% alcoholic potash for four hours. The alcohol was removed and water added to completely precipitate the unsaponifiable material, which was extracted with ether.

Examination of Unsaponifiable Matter.—The dried solution was evaporated to dryness and the residue was an orange-colored heavy viscid oil amounting to 56 g. It was dissolved in absolute alcohol (1.5 liters) and upon standing a small amount of amorphous material separated. The melting point was indefinite and suggested a mixture of hydrocarbon and a higher alcohol. By means of the phthalic acid fusion and subsequent extraction with sodium carbonate a small quantity of a hydrocarbon melting at 63–65° was isolated. Five crystallizations from ethyl acetate raised this melting point to 68–69°, the last traces running down the tube at 72°.

Calc. for $C_{35}H_{72}$: C, 85.4; H, 14.6. Found: C, 84.4; H, 14.4.

The poor melting point and low results of the combustion indicate that this was possibly pentriacontane contaminated with some alcohol.

A small quantity of sodium salt of an acid phthalic ester was isolated and boiled with alcoholic potash. A product separated which had the melting point of myricyl alcohol, 81–83°.

After the separation of the mixture of hydrocarbon and alcohol, the solu-

tion of the unsaponifiable material was concentrated and crystalline material weighing 6.1 g. was obtained. This was subjected to a fractional crystallization from alcohol. The more soluble fraction from alcohol was acetylated and the acetyl derivative, melting at 119–121°, subjected to many crystallizations from a mixture of ethyl acetate and alcohol. By this means beautiful crystalline rods of the acetate melting at 127–128° were obtained.

0.1979 g. of the anhydrous acetate made up to 20 cc. with chloroform showed a rotation of —0.76° in a 20 cm. tube, whence $[\alpha]_D^{23} = -38.5°$.

The phytosterol was regenerated from its acetyl derivative and after crystallization from alcohol it was burned. M. p. 138–139°. It was dried to constant weight at 115°.

Calc. for $C_{27}H_{46}O.H_2O$: H_2O, 4.8. Found: H_2O, 3.8.

Calc. for $C_{27}H_{46}O$: C, 83.9; H, 11.9. Found: C, 83.5; H, 12.1.

0.1732 g. of the anhydrous phytosterol made up to 20 cc. with chloroform showed at 23°, a rotation of —0.62° in a 20 cm. tube, whence $[\alpha]_D^{23} = -36°$.

The most insoluble fraction of the unsaponifiable material from alcohol yielded an acetyl derivative, crystallizing from ethyl acetate and alcohol in beautiful plates, melting at 119–120°. This was shown to be a mixture of the ordinary phytosterol ($C_{27}H_{46}O$) and another of lower carbon content. Lack of material prevented the isolation and subsequent identifications of this.

The syrupy mother liquor remaining after these substances had been isolated was freed from alcohol and subjected to a fractional distillation. It passed over between 190° to 320° at 15 mm. pressure. Most of the fractions were thick, sticky oils which did not solidify on cooling. The fraction distilling at 270° to 320° partially solidified and after several crystallizations from ethyl acetate and alcohol gave crystalline material similar to the above described mixture.

Examination of the Fatty Acids.—The alkaline solution from which the unsaponifiable matter had been extracted with ether was acidified and the liberated fatty acids were extracted with ether. The ether solution was dried over sodium sulfate and then the ether was removed and the residue distilled under diminished pressure. These boiled chiefly at 230–280° at 20 mm. The weight of the distilled acids was 36 g. and the iodine number was 110.3.

These acids were mixed with those which had been extracted with potassium carbonate solution. Two portions weighing 20.5 g. each were converted into the lead salts, which were treated with ether in the usual manner. Liquid acids equivalent to 64.7% and 66.8% of the total acids were obtained.

The lead salts of the fatty acids, insoluble in ether, were decomposed with hydrochloric acid and the solid fatty acids separated in the usual

manner. These were taken up in ether, dried over sodium sulfate and the solvent removed.

These dried fatty acids were then esterified by the method outlined by Phelps and Phelps.[1] The dried acids were dissolved in absolute methyl alcohol containing 2% hydrochloric acid and to which fused zinc chloride was added. Through this mixture during the interval of about one hour was driven absolute methyl alcohol. The crude ester was then taken up in ether extracted with potassium carbonate solutions, washed, dried and the solvent removed. By this procedure average yields of 93% of the ester were obtained.

The methyl esters of the solid acids practically all distilled under 205° at 20 mm. pressure. Most of the acid came over at 195–200° at 20 mm. pressure. These were distilled several times.

Palmitic acid melting at 61–62° was isolated.

Calc. for $C_{16}H_{32}O_2$: C, 75.0; H, 12.5; N. V., 219.1. Found: C, 75.0; H, 12.3; N. V., 210.9.

The unsaturated acids were regenerated from their lead salts soluble in ether and converted into their methyl esters. The latter were fractionally distilled 6 times under 20 mm. pressure when the following fractions were obtained:

Fraction I (b. p. —215°). This fraction was the largest containing about 80% of the acids. It gave analytical results corresponding to impure methyl linolate.

Calc. for $C_{19}H_{34}O_2$: C, 77.5; H, 11.5; I. V., 172.7. Found: C, 77.8; H, 114; I. V., 160.3.

Oxidation with potassium permanganate in the usual manner yielded a small quantity of sativic acid melting at 155–160°.

Fraction II (b. p. 215–218°). This fraction was small, amounting to about 4% of the total liquid acids. It had an iodine number of 160.3.

Fraction III (b. p. 218–222°). This fraction amounted to about 12% of the total liquid acids.

Calc. for $C_{19}H_{34}O_2$: C, 77.5; H, 11.5; I. V., 172.7. Found: C, 77.8; H, 11.3; I. V., 168.1.

The boiling point and analytical data show this fraction to be methyl linolate.

Fraction IV (b. p. 222–226°). This fraction was small, amounting to about 4%. It had an iodine number of 161.1.

The liquid acids are thus shown to consist almost exclusively of linolic acid.

The ether extract of the resin, amounting to about 3 liters, separated 54 g. of smeary resin on standing and as the extraction continued. This was filtered off. The more readily soluble part amounted to 70 g. The

[1] *Am. J. Sci.*, [4] **24**, 194–196 (1907).

green precipitate was dried and powdered and boiled with several liters of ether for days. The part insoluble in the ether was dissolved in boiling absolute alcohol and 5.5 g. remaining insoluble. Concentration of the alcoholic filtrate yielded about 6 g. further. After crystallization from dilute pyridine several times a light green semi-crystalline product was obtained which was an impure phytosterolin. With much difficulty 3.7 g. of phytosterolin melting at about 285–295°, was obtained.

Calc. for $C_{38}H_{56}O_6$: C, 72.3; H, 10.2. Found: C, 71.9; H, 10.2.

The acetate melted at 167–168°.

0.4919 g. acetate made up to a volume of 20 cc. with chloroform showed a rotation of 1.114° in a 20 cm. tube at 23° whence $[\alpha]_D^{23} = -22.6$.

The ether extract from which the crude phytosterolin had been filtered off was extracted with solutions of ammonium carbonate, sodium carbonate and potassium hydroxide, and then the ether upon evaporation contained but a slight quantity of dissolved substance. The alkaline extracts which contained quantities of chlorophyll yielded nothing of interest upon acidification.

The chloroform extract of the resin amounted to 85 g.

A further quantity of phytosterolin (2.75 g.) was obtained here. Nothing was isolated by alkaline extractions. The fraction was not glucosidic.

The ethyl acetate extract of the resin (14 g.). A small quantity (3.9 g.) of adonitol separated as the extraction proceeded. It melted at 102–3°.

Calc. for $C_5H_{12}O_5$: C = 39.5; H = 7.9. Found: C = 39.3; H = 7.8.

The alcoholic extract of the resin (90 g.). A very small amount of the toxicity was in this fraction. However, the material itself was quite toxic (0.0411 g. per 1 g. frog). An acid and an alkaline hydrolysis failed to yield anything of interest. The solution reduced Fehling's solution directly, but this reduction was increased after heating with aqueous acid.

KALAMAZOO, MICH.

[CONTRIBUTION FROM THE RESEARCH LABORATORY, HERCULES POWDER COMPANY.]

A QUANTITATIVE ESTIMATION OF ACETIC, PROPIONIC AND BUTYRIC ACIDS.

BY R. D. CROWELL.

Received November 19, 1917.

The separation of the lower acids of the acetic series from each other has been accomplished more or less satisfactorily by methods based upon the following principles:[1]

[1] Allen's "Commercial Org. Analysis," 1, 515–519 (1914). Owing to the inadequacy of library facilities it was impossible to consult the original literature on this subject.