termins the velocity of the reaction. Water retards the reaction simply by dissociating this complex, as it dissociates salts electrolytically and as it breaks up associated molecular species generally. It is important to observe that the existence in sugar solutions undergoing inversion of a special compound, an "active sugar," has been inferred from entirely different phenomena by Arrhenius.[1] The nature of this hydrolyzable complex is being studied here in connection with the role of the acid.

In conclusion, it is a pleasure to acknowledge our indebtedness to the Bache Fund of the National Academy of Sciences for a pecuniary grant in aid of these investigations. Pending the construction for us of a large instrument by Schmidt and Haensch, we used a saccharimeter placed at our disposal by Professor Alfred M. Peter, of the Agricultural Experiment Station of the University of Kentucky. We again thank Dr. Peter for his courtesy and generosity.

WORCESTER, MASS.

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

ZYGADENINE. THE CRYSTALLIN ALKALOID OF ZYGADENUS INTERMEDIUS.[2]

BY F. W. HEYL, F. E. HEPNER AND S. K. LOY.

Received December 30, 1912.

The poisonous properties of the genus *Zygadenus* have for some time been correctly ascribed to the alkaloidal substances present, but no satisfactory description of a chemically pure substance has been offered. In general, the crude alkaloidal mixture has been extracted, and to this various color reactions have been applied.

Slade[3] obtained evidence by means of such color tests which led him to conclude that the alkaloids were identical with sabadine, sabadinine, and veratralbine. Reid Hunt,[4] by similar methods, showed reason for believing that the crude alkaloidal mixture resembles veratrine. Georg Heyl,[5] working with the product of an alkaloidal assay of a California species of *Zygadenus*, ascribes a definit melting point, 134-135°, but does not go further than this statement.

It has been shown in a previous communication from this laboratory[6] that the leaves of *Zygadenus intermedius*, when assayed by gravimetrical methods, will yield between 0.3% and 0.4% of a crude alkaloidal mixture.

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[1] Arrhenius: *Z. physik. Chem.*, 4, 233 (1889).

[2] The expenses of this investigation were defrayed from the Adams fund.

[3] *Am. J. Pharm.*, 77, 262 (1905).

[4] *Am. J. Physiol.*, 6, 19 (1901).

[5] *Süddeut. Apoth. Zeit.*, 43, No. 29.

[6] THIS JOURNAL, 33, No. 2, Feb., 1911.

We have now extracted a large quantity of the leaves, and by fractionally crystallizing the crude mixture, succeeded in isolating a beautifully crystallin alkaloid, which we have termed Zygadenine. This substance melts sharply at 200–201° and gives analytical results which correspond to those required by the formula $C_{39}H_{63}NO_{10}$.

Experimental Part.

Preparation of Zygadenine.—Thirteen and one-half kilograms of dried Death Camas leaves were extracted with 33 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 maceration repeated as before. The alcoholic solution was concentrated under diminished pressure to a volume of approximately three liters. This concentrated extract was now poured into about twelve liters of distilled water, which had been acidified by the addition of 35 grams of tartaric acid. The resin was completely precipitated at this dilution. The mixture was thoroughly shaken and then permitted to stand for several days. When the black resin had settled compactly, the supernatant liquid was removed by filtration and the resin was washed with aqueous tartaric acid.

The acid filtrate, which had a reddish color, was thoroughly extracted with ether, about eleven liters being used. The aqueous solution was now rendered alkalin with sodium carbonate, and again repeatedly extracted with ether. As the extraction proceeded very slowly, some sodium hydroxide was added and the ether extraction continued. Since, after many ether extractions, the alkaloid was still incompletely removed, chloroform was substituted and the extractions were continued until the alkaline aqueous liquid was almost alkaloid-free. The last traces were removed with exceeding difficulty by extracting with amyl alcohol, but the amyl alcoholic extracts have not been investigated.

The ethereal solutions of the alkaloids were now extracted with a 5% tartaric acid solution and likewise the chloroform solution was freed from alkaloid. It was found that the addition of sodium carbonate to the tartaric acid solution precipitated a semi-solid mass, which became brittle on standing over night. However, a considerable quantity was not precipitated by this reagent, and the alkaloid remaining in solution could be extracted in part with ether, after which chloroform again extracted a further quantity. These steps, however, did not lead to separations of the individual alkaloids, as zygadenine was isolated from all three fractions, *i. e.*, from the precipitate, from the ether, and again from the chloroform extract.

As has been stated, the extractions with ether were carried out in two series, those of the second series being extracted from a more strongly alkalin liquid than in the first case. The tartaric acid solution of the tartrates, extracted from the first series of ether extracts, yielded, when again rendered alkaline with sodium carbonate, a precipitate weighing 5.7 grams; and the chloroform extract of the filtrate weighed 2.08 grams. The ether extract was not weighed. It yielded a beautiful crystallin crop of zygadenine after a series of fractional crystallizations from ether.

When the tartaric acid extract of the second series of ether extracts was rendered alkaline with sodium carbonate, a heavy precipitate of crude alkaloid was obtained. The weight, after drying in a desiccator over sulfuric acid, was 35.7 grams. When the alkaline filtrate from this precipitate was extracted with ether about 10 grams further was isolated.

Exclusive of the chloroform extract of the main solution, made after the two series of ether extractions, and exclusive also of the amyl alcoholic extract, the weight of alkaloid from 13.5 k. is 53.48 grams, or about 0.40%.

The alkaloid was isolated from the crude alkaloidal precipitate as follows: It was boiled with absolute ether in sufficient quantity to render practically all soluble. When this ether solution was allowed to evaporate spontaneously it always left a varnish and showed no tendency to crystallize. If, however, it is concentrated upon the steam bath, a hot supersaturated solution is obtained which yields a white, amorphous product after a certain concentration is obtained. This separation was accompanied by violent effervescence and bumping. The evaporation is stopped, the liquid cooled, and the amorphous material filtered off. The first crop melted at 193° and weighed about 5.6 grams. This process was repeated upon the ethereal filtrate and a second crop separated. It was much less pure, decomposing at 175°–185°. When now the filtrate was again concentrated, a third crop could be obtained, but it was a sticky, glutinous mass, which we did not attempt to purify.

It was found possible to isolate a considerable quantity further by completely removing the ether and crystallizing the residue from 95% alcohol, whereupon zygadenine separates in rhombic crystals, containing alcohol of crystallization, in which condition it is much less soluble than in the anhydrous state. The final mother liquors, upon evaporation, yielded uncrystallizable syrups.

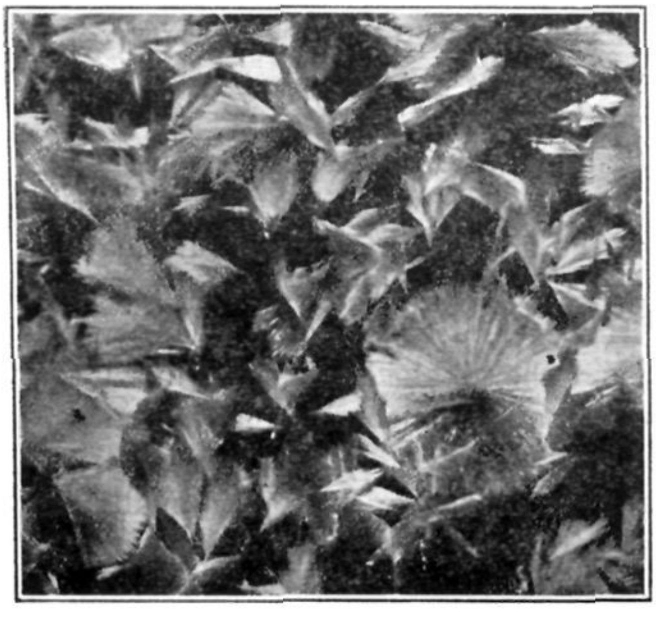

FIG. I. Zygadenine crystallized from benzene. Magnified about 480 times.

The material melting at 193° was now subjected to an extensive fractional crystallization from benzene, and in this manner a small amount of varnish was accumulated in the mother liquors and pure zygadenine melting at 200°–201°, to a red oil, was obtained in the less soluble fractions. It crystallizes in radiating clusters of shining needles from benzene (Fig. 1). It is very soluble in chloroform, but much less soluble in ligroin or ethyl acetate. For the pure alkaloid, ether is a poor solvent.

Determination of Composition.—Of the combustions described below, II, III and IV were made upon material, the preparation of which was just described. The nitrogen determinations were made upon the same material. Combustion I was made upon material isolated from the ether extract of the filtrate after precipitating with sodium carbonate. In order to test the homogeneity of the product analyzed in the first case, it was subjected to several further recrystallizations from alcohol, whereupon it was dried at 130°–140°, recrystallized again from benzene and analyzed (Combustion V).

Subst., 0.1620, 0.1425, 0.1486; CO_2, 0.3958, 0.3456, 0.3613; H_2O, 0.1292; 0.1051, 0.1183

Subst., 0.1182, 0.1187 CO_2, 0.2882, 0.2899 H_2O, 0.0947, 0.0950

Subst., 0.2819, 0.4950, 0.3846; N, 0.00667, 0.00882, 0.00775

	Calculated for $C_{39}H_{63}NO_{10}$.	Found.				
		I.	II.	III and VI.	IV and VII.	V and VIII.
C.........	66.33	66.64	66.14	66.31	66.50	66.61
H.........	9.01	8.86	8.25[1]	8.91	8.97	8.96
N.........	1.99	....	....	2.37	1.80	2.01
O.........	22.67					

Molecular weight data:[2]

	Boiling points.		Volumes. Cc.	
Alcohol..................	3.845			
Solution..................	3.891	3.886	27.2	29.4
"	3.883	3.882	30.6	32.1

M . W = KW/ΔV (where K = 1560, and W = 0.541 gram) gives 675, 701, 725 and 710. Calculated for $C_{39}H_{63}NO_{10}$, 705.5.

Properties of the Alkaloid.—Zygadenine crystallizes from alcohol in orthorhombic blocks (Fig. 2). Some of the alkaloid which had been repeatedly recrystallized from absolute alcohol was analyzed as follows:

I. 0.7256 gram substance dried to constant weight over H_2SO_4 lost at 130°–140°, 0.0828 gram.

II. 0.7345 gram subst. dried as above, lost at 140°, 0.0849 gram.

	Calculated for $C_{39}H_{63}NO_{10}.2C_2H_6O$.	Found.	
		I.	II.
C_2H_6O..........................	11.56	11.41	11.56

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[1] Not included in the average.

[2] We desire to express our thanks to Prof. McCoy and Dr. Raiford, of the Univ. of Chicago, for the molecular weight data. Another determination upon a more concentrated solution resulted as follows: 2.2867 grams subst. in 28.1 cc. alcohol gave Δ = 0.192° whence M = 661.

An acid solution of the alkaloid yields a voluminous precipitate with Meyer's reagent, and a beautiful chloroaurate is formed, when to the hydrochloric acid solution of the base, an acid solution of chloroauric acid is added. This salt is soluble in hot water and crystallizes, upon cooling, in elongated, dense prisms. With concentrated *sulfuric acid* a yellowish orange coloration is obtained, which passes over into a brilliant cherry-red. These color changes greatly resemble those of cevadine.

FIG. II. *Zygadenine crystallized from alcohol.* Magnified about 100 times.

Specific Rotation.—The alkaloid is levorotatory. 0.7028 gram dissolved in 25 cc. chloroform was found to rotate 2.7° to the left in a 2 dcm. tube. The specific rotation is therefore —48.2°.

Dr. Philip Mitchell reports upon its physiological action as follows: "Toxicologically this preparation is quite different from that of the mixed alkaloids. It shows none of the characteristic effects given by that mixture. Its behavior is, in general, very much like that of veratrine. It kills guinea pigs slowly and only in comparatively large doses, with an effect apparently due to failure of respiration, and clearly involving complete loss of muscular control. No tetanic spasms and no noteworthy effect on the heart could be observed in the guinea pigs. With frog muscle, treatment with the alkaloid dissolved in alcohol, neutralized and diluted with Ringer's solution, gave an effect like that of veratrine. A typical 'veratrine curve' was obtained."

LARAMIE, WYO.

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

STUDIES IN THE CYCLOPENTADIENE SERIES. III. CERTAIN DERIVATIVES OF *2,3-DIBENZOYL-5-NITROCYCLOPENTADIENE.*

BY WILLIAM J. HALE AND LAMBERT THORP.[1]

Received January 18, 1913.

The formation of a cyclopentadiene ring by condensation of a 1,3-

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[1] The work described in this article formed part of a thesis submitted for the degree of Doctor of Philosophy in the University of Michigan by Lambert Thorp.