ON THE ALKALOIDS OF WHITE HELLEBORE. I.
ISOLATION OF CONSTITUENT ALKALOIDS.

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Received November 1st, 1933. Published January 28th, 1934.

Since in 1819 Pelletier and Caventou(1) obtained from white hellebore, *Veratrum album* roots, a sternutatory alkaloidal body, which they called "veratria," Simon(2), Will(3), Tobien(4) and other chemists and pharmacists have studied *Veratrum album* and other allied species (*V. viride*, *V. nigrum* etc.) with results in many respects contradictory of each other.

An important contribution to the knowledge of alkaloids of *Veratrum album* was made by Wright and Luff(5), who treated the crushed dried roots with alcohol containing tartaric acid and showed that they contained at least five different alkaloids. Jervine, $C_{26}H_{37}NO_3$; pseudojervine, $C_{29}H_{43}NO_7$ and rubijervine, $C_{26}H_{43}NO_2$ were well-defined crystallisable nonsternutatory bases and a fourth, veratralbine, $C_{24}H_{43}NO_5$ was amorphous and nonsternutatory whilst the fifth, veratrine, $C_{27}H_{53}NO_{11}$ was highly sternutatory.

Seven years later Salzberger(6) was able to isolate besides the abovementioned three crystallisable bases, jervine, pseudojervine and rubijervine, two new minor alkaloids: protoveratrine, $C_{32}H_{51}NO_{11}$, an extremely powerful poison, and protoveratridine, $C_{26}H_{45}NO_8$, in a very minute amount (ca. 0.001 per cent.). In isolating these alkaloids two methods of extraction were followed. One, the baryta method, is relatively rapid and gives jervine, rubijervine and protoveratridine but no protoveratrine. The other, the metaphosphoric acid method, yields protoveratrine and pseudojervine with small amounts of jervine and rubijervine.

The constitutions of these alkaloids, however, have not yet been investigated. Moreover, the material produced in this country does not appear to have been studied previously and this group of medical plants(7) occurs in the various species indigenous to this island, Hokkaido, and is fortunately found everywhere in the field. *Veratrum grandiflorum Loes. fil.*(7), former-

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(1) *Ann. Chim. Phys.*, (2), **14** (1819), 69.
(2) *Pogg. Ann.*, **41** (1837), 569.
(3) *Ann. Chem. Pharm.*, **35**, 116.
(4) *Pharm. J. Trans.* April 13, (1878) p. 808.
(5) *J. Chem. Soc.*, **33** (1878), 338; **35** (1879), 405, 421.
(6) *Arch. Pharm.* **228** (1890), 462.
(7) K. Miyabe and Y. Kudo, *J. Coll. Agric. Hokkaido*, **26** (1932), 311.

ly described as *Veratrum album*, is also widely distributed in the mountainous districts of Hondo.

The roots for this investigation were collected at Nopporo, a suburb of Sapporo, in the autumn. As in the course of the preliminary test of extraction the odour of a comparatively lower member of fatty acids, an indication of saponification, was recognized, acid or alkaline extraction applied by former authors was avoided.

The finely powdered roots were percolated with alcohol at room temperature, distilling off the solvent under reduced pressure, and shaking the faintly acid aqueous extract with light petroleum to remove oily and resinous matters. On basifying the aqueous liquor with ammonia or sodium bicarbonate brown basic substances were separated and collected, **portion A.** The filtrate, containing some more alkaloids, was extracted with chloroform after saturation with ammonium sulphate, and amorphous bases were obtained, **portion B.** The crude bases thus obtained were next treated with the usual organic solvents for fractional crystallisation and it was found that ethyl acetate and carbon tetrachloride were the solvents for the purpose but not quite suitable. Attention was then directed to the fractional precipitation and the bases, portion A as well as B, were dissolved into dilute acetic acid and divided into six fractions with ammonia. On neutralisation of each fraction of portion A and B with hydrochloric acid, the first three of portion A gave only jervine hydrochloride in a crystalline condition, but no crystalline alkaloid was obtained from each fraction of portion B. Unfruitful attempts were made to obtain a crystalline substance from the amorphous part by formation of salts, using a variety of acids, organic as well as inorganic.

Since pseudojervine, rubijervine, protoveratrine and protoveratridine which are stated by former authors to have been isolated from European sources in a crystalline condition could not be obtained, the method of isolation after Salzberger(6) was repeated.

It was found, however, that the material used for this investigation was different from the European, and jervine was so far the only crystalline alkaloid in the material.

The amorphous base of portion B was next hydrolysed in a sealed tube with the expectation of obtaining a crystalline hydrolysed base but the results were unsuccessful. On heating with water under pressure or with alcoholic potash, the amorphous base underwent hydrolysis into another amorphous base and angelic acid. On hydrolysis with concentrated hydrochloric acid, the same base gave tiglic acid. The hydrolysis of a veratrum alkaloid, cevadine or crystallised veratrine, is described in the literature, with different results. Cevadine was first hydrolysed by Wright and Luff⁽⁸⁾ who obtained tiglic acid on hydrolysis with alcoholic soda or with water under pressure but Bosetti⁽⁹⁾ secured angelic acid by alcoholic baryta. The latter suggested that angelic acid was the first product of the hydrolysis of veratrine, and is subsequently converted into tiglic acid. Some years later Ahrens⁽¹⁰⁾ repeated the hydrolysis with alkali and concentrated hydrochloric acid and obtained angelic acid in the case of alkali and tiglic acid in the other case, thus supporting the suggestion of Ahrens.

Freund and Schwarz⁽¹¹⁾, however, obtained angelic acid as well as tiglic acid on hydrolysing the same alkaloid with alcoholic potash. Recently Horst⁽¹²⁾ tried the hydrolysis, passing hydrogen chloride into an alcoholic solution of cevadine, and isolated ethyl tiglate, which, on hydrolysis, gave tiglic acid. It is stated that the tiglic acid is not a product from the angelic acid first formed.

On repeating the hydrolysis of the crude base from *Veratrum grandiflorum Loes. fil.* after Horst, tiglic acid was obtained; it appears that angelic acid is the product first formed in the hydrolysis as has been suggested already by Bosetti⁽⁹⁾ and Ahrens⁽¹⁰⁾ because of the easiness with which angelic acid is convertible into tiglic acid. On the other hand, tiglic acid is so stable that isomerisation did not take place on treatment with water at 120°C. in a sealed tube.

Experimental.

Extraction of the Alkaloids. One part of finely powdered material, collected at Nopporo in October and dried in the sun, was kept in a percolator with two parts of alcohol (95%) and was allowed to stand at room temperature for several days. During the extraction it was shaken at least once a day. The solvent distilled off under reduced pressure, the temperature of the water-bath being kept under 50°C., until practically the whole had been removed, leaving a semi-solid residue. The extraction was repeated with the recovered alcohol. On treating the residue with water and light petroleum (b. p. 40–60°C.) a considerably large amount of dark resinous matter separated and was filtered off. The extract, being faintly acidic, was further shaken with light petroleum several times and finally bubbled with air, when some more resinous matter separated. After filtering off the

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(8) *J. chem. Soc.*, **33** (1878), 335.
(9) *Arch. d. Pharm.*, **221** (1883), 87.
(10) *Ber. Deutsch. chem. Gesell.*. **23** (1870), 2703.
(11) *Ber.*, **22** (1899), 800.
(12) *Chem. Zeit.*, **22** (1902), 334.

latter, the brown solution was rendered slightly alkaline with a solution of 6 N-ammonium carbonate. The yellowish brown bases separated were collected, **portion A,** washed, and dried over fresh conc. sulphuric acid in a vacuum desiccator. With ammonium sulphate the mother liquor was salted out and shaken with chloroform. The chloroform solution was dried over anhydrous sodium sulphate, the solvent distilled off thoroughly, leaving an amorphous solid, **portion B.** From the dark resinous matter separated on dilution of the semi-solid residue with water some more alkaloids could be extracted best with dilute acetic acid. The light petroleum which had been used to remove the oily substance from the aqueous extract gave also a little more of the alkaloids by treating with 1 per cent. hydrochloric acid. The yield of the crude alkaloids is 1.2–1.5% referring to the material as shown in the following table:

Material: 12 kg.; alcohol (95%) 24 L.

Number of extraction		I	II	III	IV
Days percolated		3	6	6	10
Aqueous extract	portion A in gr.	45	23	19.5	5.5
	portion B in gr.	26.0	16.5	17.0	6.2
Acetic acid extract	portion A in gr.	7.1	2.6	0.8	0
	portion B in gr.	7.5	1.5	0	0
Hydrochloric acid extract	portion A in gr.	1.5	0	0	0
Total yield in gr.		87.1 +	43.6 +	37.3 +	11.7 = 179.7

Isolation and Purification of the Bases. Crude alkaloids obtained by the foregoing method are easily soluble in methyl and ethyl alcohol and are soluble in ethyl acetate and acetone, but very sparingly soluble in ether, chloroform, light petroleum, benzene and carbon tetrachloride.

Attempts to fractionate individuals by taking advantage of differing solubilities in solvents were unsuccessful. With carbon tetrachloride jervine was isolated in a crystalline condition but was not satisfactory.

A suspension of the crude alkaloids, portion A (10 gr.) in carbon tetrachloride (1000 gr.) was boiled under a reflux condenser for an hour, filtered, and the filtrate concentrated. On allowing to stand overnight in the ice-chest a brown crystalline powder separated which was collected. Yield was 0.79 gr. The insoluble part was treated with the recovered solvent repeatedly, and the crystalline powder (0.25 gr.) was obtained. On recrystallisation of the crystalline substance thus obtained with methyl alcohol, colourless needle crystals, melting at 242°C., separated. These were collected and proved to be jervine.

The insoluble part (7.5 gr.) was treated with solvents but was not crystallisable and was next made acidic with alcoholic hydrochloric acid. On keeping in the cold, jervine hydrochloride crystallised in colourless stout prisms which were collected, washed with alcohol, and dried. Weight 0.03 gr.; m. p. 308°C. (with decomposition). The crude base, portion B, was also treated with carbon tetrachloride as in the former case. On extraction of the portion B (5 gr.) with the solvent (500 gr.) a comparatively soluble matter (2.85 gr.) was obtained but could not be obtained in a crystalline condition. This fraction soluble in carbon tetrachloride was then converted into hydrochloride, hydrobromide, oxalate, benzoate and acetate, but did not crystallise. From the fraction of portion B insoluble in carbon tetrachloride no crystalline individual was obtained.

Separation by Fractional Precipitation. *Portion A*. Crude alkaloids (40 gr.) were dissolved into 0.25 N-acetic acid (240 c.c.) and were fractionarily precipitated with 0.5 N-ammonium hydroxide solution (120 c. c.) dividing into five parts. The first fraction was small in amount, coloured brown and mixed with resinous matter, but the amounts of the second fraction and the third were increased rapidly. The weights of the fourth and the fifth fraction were decreased gradually. The mother liquor of the fifth fraction was salted out with ammonium sulphate and was shaken with chloroform. The chloroform solution was washed with a small amount of water and dried over anhydrous sodium sulphate. The solvent was distilled off as completely as possible, leaving a solid mass. This was the sixth fraction.

Each fraction thus obtained was then in turn converted into hydrochloride with 0.1 N-alcoholic hydrogen chloride. On allowing to stand for a while jervine hydrochloride separated from the first, second and third fractions, was collected and dried. Yield 6.71 gr. or 0.13% of the material. In the following table the yield of the salt from each fraction is shown.

Number of fraction	I	II	III	IV	V	VI
Precipitates in gr.	1.62	15.35	7.90	0.77	0.67	10.00
Jervine hydrochloride in gr.	0.55	4.15	2.11	0	0	0

On concentration of the mother liquor of jervine hydrochloride some more salt separated which was removed as completely as possible and the uncrystallisable hydrochlorides of each fraction, which are the greater part of the crude bases, were set free. From these parts neither a free base nor a salt such as sulphate or aurichloride was obtained in a crystalline condition.

Jervine Hydrochloride. The salt is very sparingly soluble in absolute alcohol or water but soluble in dilute alcohol and crystallises in colourless prisms which melt at 308°C. with decomposition.

According to Salzberger(6) jervine hydrochloride crystallised from alcohol contains two molecules of water of crystallisation but it is now shown that the salt crystallises with one molecule of water and one molecule of alcohol from less diluted alcohol (e. g. 95%) and with different molecules of the solvents from more diluted alcohol (e. g. 75, 50 and 30% alcohol).

The melting points of each modification are practically the same—308°C. with decomposition—but the crystal forms of the modifications are slightly different.

Anal.: 4.329 mg. of air-dry crystals lost at 125°C. and 15 mm. 0.529 mg.
4.219 ,, ,, 0.588 mg.
Found $H_2O{\cdot}C_2H_5OH = 12.23\%$.
$C_{26}H_{37}O_3N{\cdot}HCl{\cdot}H_2O{\cdot}C_2H_5OH$ requires 12.51%.
Air-dry salt = 4.602; $CO_2 = 10.979$; $H_2O = 3.776$ mg.
Found: C = 65.07; H = 9.18%.
$C_{26}H_{37}O_3N{\cdot}HCl{\cdot}H_2O{\cdot}C_2H_5OH$ requires C = 65.65; H = 9.06%.

The salt dried at 125°C. in vacuo was analysed with the following results.

Anal.: Subst. = 4.065, 3.800, 4.254; $CO_2 = 10.340, 9.705, 10.820$;
$H_2O = 3.140, 2\,878, 3.231$ mg.
Subst. = 4.627 mg. $N_2 = 0.124$ c.c. at 17°C. and 766 mm.
Subst. = 4.447; AgCl = 1.442 mg.
Found: C = 69.33, 69.66, 69.37; H = 8.57, 8.47, 8.50; N = 3.18; Cl = 8.02%.
Calc. for $C_{26}H_{37}NO_3HCl$: C = 69.68; H = 8.55; N = 3.13, Cl = 7.92%.

The hydrochloride crystallised from 75, 50 and 30 per cent. alcohol gave ethyl iodide on treating with hydroiodic acid by the micro-Zeisel method.

The alcohol in the hydrochloride, crystallised from 30 per cent. alcohol, was determined as ethyl iodide.

Anal.: 4.383 mg. of air-dry crystals lost at 125°C. and 20 mm. 0.509 mg.
Found: $H_2O{\cdot}C_2H_5OH = 11.61\%$.
Subst. = 4.238; AgI = 1.742 mg.
Found: $C_2H_5OH = 8.06\%$.

Jervine, Free Base. The pure hydrochloride dissolved in dilute alcohol was treated with ammonia, when the free base was separated, collected, washed and dried. Jervine crystallised from dilute alcohol in colourless needles taking two molecules of water of crystallisation, and m. p. 240–241°C.

Anal.: 4.484, 4.552 mg. air-dry crystals lost at 125°C. and 15 mm. 0.367, 0.373 mg.
Subst. (air-dry crystals) = 4.411, 4.249; $CO_2 = 11.290, 10.869$; $H_2O = 3.660, 3.467$ mg.
Found: $H_2O = 8.19$; C = 69.81, 69.76; H = 9.28, 9.13%.
Calc. for $C_{26}H_{37}NO_3{\cdot}2H_2O$: $H_2O = 8.05$; C = 69.75; H = 9.24%.

The anhydrous substance was also analysed.

Anal.: Subst. = 4.195, 4.292, 4.288; CO_2 = 11.635, 11.889, 11.876; H_2O = 3.470, 3.555, 3.575 mg.
Subst. = 4.270 mg.; N_2 = 0.120 c.c. at 16°C. and 764 mm.
Subst. = 4.765 mg.; N_2 = 0.143 c.c. at 18°C. and 766 mm.
Found: C = 75.64, 75.55, 75.54; H = 9.26, 9.27, 9.33; N = 3.34, 3.55%.
Calc. for $C_{26}H_{37}NO_3$: C = 75.86; H = 9.07; N = 3.41%.

Jervine contains no methoxyl group and is laevorotatory, having the following specific rotation which has not been described previously.

$$[\alpha]_D^{19^\circ} = \frac{-0.30\times 100}{0.169\times 1} = -177.5^\circ \text{ (in absolute alcohol).}$$

Jervine Acetate. This salt was prepared with the object of obtaining an easily soluble salt but it was very unstable against water or heat. On dissolving in water or heating in vacuo the free base was recovered instantly in a pure condition. Jervine in absolute alcohol was neutralised with acetic acid in the same solvent and the resulting solution was evaporated in the desiccator over concentrated sulphuric acid and finally over potassium hydroxide, leaving a colourless crystalline mass which melted at 234–238°C., indefinitely.

Attempts to recrystallise the salt from water, dilute alcohol, alcohol and ether, and other usual organic solvents were made with unfruitful results owing to the easiness with which the acetate decomposed into the original base.

Anal.: 10.427 mg. of air-dry crystals lost at 125°C. and 15 mm. 1.424 mg.
Found; $CH_3COOH,\frac{1}{2}H_2O = 13{\cdot}66\%$.
$C_{26}H_{37}O_3N{\cdot}CH_3CO_2H{\cdot}\frac{1}{2}H_2O$ requires 14.37%.
Subst. = 3.718, 3.461; CO_2 = 9.516, 8.855; H_2O = 2.784, 2.572 mg.
Found: C = 69.82, 69.78; H = 8.38, 8.31%.
Calc. for $C_{26}H_{37}O_3N{\cdot}CH_3CO_2H{\cdot}\frac{1}{2}H_2O$: C = 69.95; H = 8.81%.

The salt is also laevorotatory:

$$[\alpha]_D^{20^\circ} = \frac{-0.55\times 100}{0.4339} = -126.8^\circ \text{ (in absolute alcohol).}$$

Portion B. This was also fractionarily precipitated and converted into hydrochlorides as in the case of portion A, but neither base nor hydrochloride could be obtained in a crystalline condition.

Number of fraction	I	II	III	IV	V	VI
Precipitates in gr.	1.00	7.15	3.85	1.60	6.0	15.50
Jervine hydrochloride	0	0	0	0	0	0

Hydrolysis of the Crude Base, Portion B. *With Water.* The crude base (5.0 gr.) was heated in a sealed tube with water (100 c. c.) at 120°C. for five hours when an acidic solution, coloured dark brown and partly charred, was obtained. To the filtered solution 1N.–sulphuric acid (10 c. c.) was added and subjected to steam distillation. The distillate was neutralised with caustic soda and evaporated, leaving a colourless mass (0.54 gr.). From the salt the free acid was prepared, and was crystallised from water in colourless prisms, melting at 46°C., and was identified as angelic acid.

Silver salt was prepared, melting at 196°C. with decomposition.

Subst. = 4.692 ; Ag = 2.45 mg. Found : Ag = 52.02%.
Calc. for $C_5H_8O_3Ag$: Ag = 52.17%.

With Alcoholic Potash. Crude base (20 gr.) was dissolved in alcohol (80 c. c.) and was heated on the water-bath for twenty minutes with caustic potash (10.4 gr.) in alcohol (80 c. c.). On cooling, the reaction product was separated, filtered. The filtrate was saturated with carbon dioxide, separating potassium carbonate. The latter was removed and the solution was concentrated under diminished pressure, leaving a brown powder. After dissolving in water, it was filtered, acidified with sulphuric acid, and extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate and the solvent was distilled off, when a crystalline substance remained. The acid crystallised from water in colourless prisms, melting at 45°C., and was identical with angelic acid. Yield after recrystallisation 0.14 gr.

With Concentrated Hydrochloric Acid. The hydrolysis was made after Ahrens.(10) The same crude base (20 gr.) was heated on the wire gauze for an hour with concentrated hydrochloric acid (50 c. c.). At the early stage of the reaction a reddish brown substance separated, which converted into a dark homogeneous solution on further heating. The reaction mixture was cooled, treated with water (360 c. c.) and was then shaken with ether. After drying the ethereal solution the solvent was distilled off, leaving a crystalline substance (0.31 gr.). This was recrystallised from water in colourless plates, m. p. 64°C., and was identified as tiglic acid.

3.767 mg. substance required 1.86 c.c. 1/50 N–NaOH.
Calc. for $C_5H_8O_2$,, 1.88 c.c. ,, .

With Alcoholic Hydrogen Chloride. The same crude base was hydrolysed after Horst(12) as follows. Crude base (20 gr.) was dissolved in absolute alcohol (75 c. c.) and the resulting solution was boiled on the water-bath for four hours. In the course of heating dry hydrogen chloride was passed through the solution. The alcohol was distilled off when a reddish brown liquid (ca. 1.2 c. c.), having a pleasant smell, together with a crystalline substance, remained. The latter was identified as tiglic acid ; the former was treated with alcoholic potash, the hydrolysis being completed by heating for a short time. On distilling off the solvent the residue was acidified, extracted with ether and the ethereal was dried. From the ethereal solution tiglic acid was obtained, melting at 64°C.

Tiglic acid (10 mg.) was heated with water (0.5 c.c.) in a sealed tube at 120–130°C. for five hours. On cooling tiglic acid (4.5 mg.) separated, and was recovered, m. p. being 61–63°C.

Attempts were made to obtain the hydrolysed base in a crystalline condition but neither the free base nor the salt, hydrochloride, hydrobromide, or perchlorate, could be obtained in a pure condition.

The hydrolysis of each fractionated part of the portion B also gave the same results.

The Metaphosphoric Acid Process for Extraction after Salzberger. After treating the finely powdered material (14 kg.) with ether to remove the oily substance, it was percolated with alcohol (80%) at room temperature. The percolate was concentrated under reduced pressure, the bath temperature being kept below 40°C., when a syrupy substance remained. The latter was further shaken with light petroleum (b. p. 40–60°C.), treated with 0.5 N.-acetic acid, and was then filtered. To the filtrate powdered metaphosphoric acid was added until no further precipitation occurred. The yellow amorphous precipitates, which should contain jervine and rubijervine, were collected, dried and powdered. A suspension of this powder in alcohol was treated with ammonia and was then acidified with sulphuric acid. On warming on the water-bath the sulphate of jervine, which is very sparingly soluble in water, was collected. The mother liquor was again basified with ammonia and was next extracted with ether, but rubijervine could not be found.

The filtrate of the yellow amorphous precipitate was repeatedly extracted with ether and then with chloroform.

On evaporating the ethereal solution a small amount of crude jervine remained but not protoveratrine.

The chloroform solution was also concentrated under reduced pressure, the bath temperature being kept under 40°C., leaving an amorphous substance. The latter was crystallised from absolute alcohol and was identified as jervine instead of pseudojervine.

We desire to express our great indebtedness to Mr. K. Akagi, the Director of the Industrial Experiment Station at Kotoni near Sapporo and Mr. K. Hayashi, the chief chemist of the institute, for affording us facilities for the investigation, and to Professors K. Miyabe and Y. Yamada for the suggestions on the botanical sides.

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