ON THE ALKALOIDS OF WHITE HELLEBORE. II. ISOLATION OF ALKALOIDS FROM THE SO-CALLED RESINOUS MATTERS.

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In the previous communication(1), a method of isolating alkaloids from the roots of white hellebore was described, but no account was given of the resinous matters soluble in alcohol but insoluble in water nor in dilute acetic acid. The former authors also laid aside, and did not investigate, this part of alkaloids, considering it as resin(2), as resinous substances(3) or as "unlösliche Bestandteile"(4). Our subsequent investigation of the so-called resinous matters has shown the presence of another series of alkaloids.

The resinous matters were treated with dilute acetic acid to remove the soluble part, which gave alkaloids similar to those described in Part I on basifying with ammonia. This operation of extraction was very tedious, so that it was repeated until the yield of the bases amounted to 0.5% of the starting material.

The brown powder, insoluble in dilute acetic acid, was then treated with ether continuously and some oily matters as well as phenolic substances were removed. The latter were proved to consist chiefly of a dihydric phenol. Unfruitful attempts were made by using various solvents to obtain a crystalline substance from the powder. On treatment with dilute alkali the brown powder was decomposed into bases freely soluble in dilute acetic acid, and acidic substances with angelic acid and an acid melting at 102°. These acids, however, were rather small in amount and the acidic substances were found to be phenolic acids sparingly soluble in ether. The crude bases thus obtained could be crystallised from dilute alcohol but isolation of the components in pure condition was unsuccessful. Then they were divided into seven fractions by fractional precipitation, as done in Part I. On neutralizing each fraction with hydrochloric acid in alcohol, the first three gave jervine hydrochloride in crystalline form. From the fourth fraction some more hydrochloride could be obtained.

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(1) This Bulletin, **9** (1934), 15.

(2) Wright and Luff, *J. Chem. Soc.*, **35** (1879), 406.

(3) C. Pehkschen, *J. Chem. Soc.*, **90** (1891), 88; *J. Pharm.*, (5), **22**, 265; *Pharm. Zeit. Russ.*, **29**, 339.

(4) Salzberger, *Arch. Pharm.*, **228** (1890), 462.

The so-called resinous matters in the roots of *Veratrum grandiflorum Loes. fil.* may, therefore, be considered as a mixture of salt-like substances, consisting of alkaloids, acids, and free phenolic substances. The chief constituents of the salt-like substances are probably molecular compounds of alkaloids and phenolic compounds, while the acids, soluble in water, are combined with the alkaloids as esters.

Experiments concerning the constitution of the phenolic compounds and the isolation of other alkaloids in crystalline state are in progress.

Experimental.

Treatment of the so-called resinous matters with dilute acetic acid. After the extraction(5) of the alkaloids from the powdered roots (12 kg.) with alcohol (95%) the solvent was distilled off under reduced pressure; a semi-solid residue was obtained. On treating the latter with water and light petroleum (b.p. 40–60°) a comparatively large amount of dark resinous matters, insoluble in water, separated and were collected. They were then digested successively with dilute acetic acid and with water to remove soluble basic substances and alcohol that had been absorbed. The brown powder thus obtained was next suspended in three parts of dilute acetic acid, and the mixture was stirred all day long and filtered. On basification of the filtrate with ammonia, free alkaloids were separated, which were collected, washed, and dried. The yield was 9.1 g. The amounts of the crude alkaloids obtained in each treatment were as follows:

Number of extraction	I	II	III	IV
Basic substances in g.	2.8	2.3	2.1	1.9

As may be seen from the table extraction proceeds very slowly, so that it was given up when the yield of the bases reached 0.5% of the material. At the later stage of the treatment the insoluble powder appeared to be partly decomposed by the dilute acetic acid. The yield of the insoluble powder was 302 g. or 2.5% of the roots.

The powder is sparingly soluble in water, light petroleum, and benzene; partly soluble in carbon tetrachloride, ether, acetic ester, and acetone; freely soluble in methyl and ethyl alcohol, and glacial acetic acid. When the powder is treated with dilute alcohol, in which it is sparingly soluble, it becomes viscous and resinous in appearance but can be restored by suspension in water, the absorbed alcohol being lost.

Alkaloids extracted with dilute acetic acid. This part of the alkaloids may be considered as a mixture of the bases described as portion A in Part I and those obtained from the insoluble part through hydrolysis.

Crude bases (34 g.) were dissolved in 0.5 N acetic acid (108 c.c.), diluted with water (108 c.c) and divided into five parts by fractional precipitation with 0.5 N ammonium hydroxide. The first fraction was small in amount and brown in colour, but the amounts

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(5) Compare Part I, this Bulletin, **9** (1934), 17.

of the second and third fractions were larger. The amounts of the fourth and fifth fractions were again small. To the mother liquor of the fifth fraction an excess of ammonia was added, when some more precipitates separated and were collected as the sixth fraction. The filtrate of the sixth fraction was saturated with ammonium sulphate and was extracted with chloroform. On working up the chloroform solution the seventh fraction was obtained. Each fraction thus obtained was then dissolved in alcohol and treated with hydrochloric acid in the same solvent. On being allowed to stand for a while, a crystalline hydrochloride, melting at 308° with decomposition, separated from the first, second, third, and fourth fractions. This was collected and dried; the yield was 5.6 g. or 16% of the crude bases. The salt (5.6 g.) was suspended in hot alcohol (90%, 62 c.c.) and was treated with sodium carbonate; on cooling the solution the freshly liberated base separated in colourless needles. It was collected and recrystallised from alcohol; it melted at 242–243°, and was proved to be jervine by mixed melting point with an authentic specimen. (Found: C, 75.70; H, 9.25. Calc. for $C_{26}H_{37}NO_3$: C, 75.86; H, 9.07%. $[\alpha]_D^{21°} = -150°$ in absolute alcohol.)

In the following table the amounts of each fraction as well as the yield of the salt are shown.

Number of fraction	I	II	III	IV	V	VI	VII
Precipitate in g.	0.3	14.8	5.3	0.1	0.1	0.4	1.5
Jervine hydrochloride in g.	0.1	4.2	1.3	trace	0	0	0

Treatment of the part insoluble in dilute acetic acid with ether. In order to remove fats and oils, free phenolic substances, and free alkaloids, the brown amorphous powder was treated first with dilute acetic acid and subsequently with ether for 26 days continuously. In the earlier stage of the process extraction proceeded fairly rapidly and a yellowish brown crystalline powder was obtained but its amount decreased gradually as the days passed. In the latter stage of the extraction brown viscous substances were obtained. After 10 days 0.5% of the material was extracted in 24 hours, while on the 24th day 0.1%. Total extract in 26 days was 55 g. or 18% of the material. On treatment of the extract with light petroleum (b.p. 40–60°) about 10 g. or 19% of fats and oils were removed, leaving a yellowish brown fine powder. The latter contained a trace of nitrogen, and was sparingly soluble in sodium bicarbonate, partly soluble in sodium carbonate, and almost perfectly soluble in caustic alkali. In water, light petroleum, chloroform, and benzene, it was difficultly soluble while freely soluble in methyl, ethyl alcohol, and acetic ester. It was crystallised from dilute alcohol in plates melting at 262° with decomposition. Its alcoholic solution gave a dark brown colouration with ferric chloride; it decolourised bromine water and potassium permanganate. On dissolving it in concentrated sulphuric acid it developed a colouration similar to the case of bromine water.

Decomposition of the part insoluble in dilute acetic acid with alkali. The amorphous brown powder (247 g.) purified as described above was treated with dilute alkali (1000 c.c.) when the solution rapidly turned dark brown in contact with the air owing to the absorption of oxygen, while the compact brown powder passed into lighter, coarse, floating *bases*. After a while they were collected, washed, and dried in the air. The yield was 137 g. or 1.1% of the roots. The filtrate was saturated with carbon dioxide, when brown *phenolic substances* separated, which were collected, washed, and dried. On being treated with dilute sulphuric acid some more acidic substances were obtained. The yield of the phenolic and acidic substances altogether was 58 g. Lastly the filtrate was treated with ether continuously and *soluble acids* were extracted (ca. 26 g.). These phenolic and acidic substances rapidly turned dark brown in contact with the air through autoxidation either in an alkaline solution or in a solid state with a trace of alkali and moisture, but were comparatively stable if they were quite free from alkali as well as from moisture.

Phenolic substances. The brown phenolic substances contained no nitrogen and were comparatively difficultly soluble in ether; they gave off carbon dioxide on pyrogenetic treatment and appeared to be a phenolic acid.

Soluble acids. Among these acids angelic acid was detected. Another acid, melting at 102°, was very small in amount and was not further investigated.

Alkaloids. The bases obtained from the brown powder insoluble in dilute acetic acid had a characteristic odour and were partly soluble in benzene, ether, chloroform, acetic ester, and acetone, but sparingly soluble in light petroleum. In methyl and ethyl alcohol they were freely soluble.

Crude bases (88 g.) were dissolved in 0.5 N acetic acid (440 c.c.), the solution, after filtered from the insoluble matter, was diluted with water (440 c.c.), and the bases were fractionally precipitated. The results were similar to the other case of the fractional precipitation. Each fraction was treated with alcoholic hydrochloric acid, and jervine hydrochloride was isolated from the first to the fourth fraction; the yield was 32.8 g. or 37% of the crude bases, and 0.43% of the material. Taking into account the salt in Part I, the total yield of jervine hydrochloride amounted to 0.6% of the roots.

Number of fraction	I	II	III	IV	V	VI	VII
Precipitate in g.	4.0	25.9	40.0	1.4	0.6	0.7	1.3
Jervine hydrochloride in g.	1.5	12.5	18.8	trace	0	0	0

On treating the salt with sodium carbonate the free base was liberated and was crystallised from alcohol in colourless needles, melting at 242–243°, identified with jervine.

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