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iments, i. e. $\frac{88+62}{2}=75$; the value of $\Delta\ P$ between observations 2 and 3 is therefore,

$$\Delta\ P = \Delta\ x_d \frac{dP}{dx_d} = (0.0104-0.0067) \times 75 = 0.35\,.$$

The values of $\frac{dP}{dx_d}$ and ΔP thus obtained are given in table 6; also, for comparison, the values of $\Delta\ P$ obtained by direct experiment.

TABLE 6.

Between observations	$\frac{dP}{dx_d}$	$\Delta\ P$ calculated	$\Delta\ P$ found
No. 2 and No. 3	75	0.3 m.M.	0 m.M.
3 » 4	30	0.1	1
4 » 5	— 149	— 0.5	0
5 » 6	— 425	— 0.2	0
6 » 7	— 1190	— 1.3	0
7 » 8	— 2454	— 3.4	— 3
8 » 9	— 3618	— 18.5	— 21
9 » 10	— 3302	— 73.9	— 73

As may be seen from the table, ΔP calculated and ΔP found agree satisfactorily; the difference are smaller than the experimental errors which may amount to several m.ms.

Chemistry. — "*Echinopsine, a new crystalline vegetable base*".

By Dr. M. GRESHOFF (Communicated by Prof. A. P. N. FRANCHIMONT).

(Read April 21, 1900).

Of late years alkaloids have been discovered in plantfamilies which, previously, had been made but little the subject of phyto-chemical studies, and in which, at any rate, no vegetable bases had been found or even suspected. So, for instance, in the large family of the *Compositae*, which comprises about one-tenth part of all the phanerogamia, with more than 800 genera.

The writer has been engaged for many years in the systematic study of alkaloidal distribution in plants, also in this family,[1] and

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[1] Compare: *On the distribution of alkaloids in the family of the Compositae.* Ned. Tijdschr. v. Pharm., Mei 1900, blz. 137. In this article 50 alkaloid-containing genera are summarised, mostly the result of my own investigations.

has now the opportunity to present the meeting with at least one of his new compositae-alkaloids in a pure condition, and to give a description of the same.

First of all, some particulars about the botanical origin.

The genus *Echinops* L. (=*Echinanthus* Neck., *Echinopus* Tourn., *Sphaerocephalus* L.) belongs to the division *Tubuliflorae-Cynareae* of the Compositae. These *Cynareae* are divided into four groups: *Echinops*, *Carlina*, *Carduus* and *Centaurea*, all plants popularly known as thistles; some are characterised, from a chemical standpoint, by containing alkaloids, glucosides, bitter principles and pigments; a few yield hydrocyanic acid.

The group *Echinops* only contains this genus itself, and *Acantholepis orientalis* Less., a plant from the steppes of Central-Asia. *Echinops* numbers about 60 species, also mostly Central-Asiatic herbs with alternate, frequently thorned leaves, and all species characterised by having capitula. To the West, the *Echinops* territory extents over the whole of the South of Europe and the coasts of the Mediterranean, to the East as far as Japan; some species are also natives of tropical Africa. In Germany, *E. sphaerocephalus* L. grows wild; no species is found wild in Holland. In that country various kinds are, however, cultivated as ornamental plants, on account of the robust stature and the beautiful large flower heads from which the genus derives its name of "ballthistle" (the latin name is composed of *echinus*, hedgehog and *ops*, eye or appearance). The flowers are sometimes light blue *E. Ritro* L., or dark blue *E. bannaticus* Roch.- The genus is divided by botanists into 7 sections; compare Engler u. Prantl, *Natürliche Pflanzenfamilien* IV, 5, p. 313. The species are mostly described in Boissier's *Flora orientalis* and also by Bunge, *Bull. de l'Académie de St. Pétersbourg* VI, 390. My investigation extents over 15 species from different sections [1]) which all were found to contain echinopsine, so that there is reason to believe that the presence of this alkaloid is a general characteristic of the *Echinops*-species.

On the use of *Echinops* in popular medicine and in toxicology, a question revived by the discovery of the powerful Echinopsine, not much information is at my disposal. Different species, such as *E. Ritro* L., *dahuricus* Fisch.,

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[1]) This is perhaps the proper place to state the source of the important material of my investigation and to thank those who provided me with the same. From the botanical garden at Leiden, I received through the care of Mr. E. Th. Witte, hortulanus, *E. Ritro* L. and *E. niveus* Wall. Of the first plant, the firm Haage u. Schmidt at Erfurt provided me with the 10 kilograms fruits, which have served for the preparation of a larger quantity of echinopsine, than the supply from the botanical garden allowed. I also got from the same source *E. sphaerocephalus* L., *E. exaltatus* Schrad., *E. paniculatus* Jacq. and *E. syriacus* Boiss. On a holiday tour in Sweden in Aug. 1899 I noticed in the excellently kept botanical garden of Lund and Upsala some other varieties cultivated there. In Lund, I collected leaves of *E. dahuricus* Fisch., *E. bannaticus* Roch., *E. platylepis* Trautv. and *E. microcephalus* Sibth.; afterwards I received from there seed of *E. globifer* Janka and of another species which according to Dr. Sv. Murbeck was, probably, *E. commutatus* Jur. From Lund, the hortulanus Mr. Fr. Petterson forwarded me beautiful material from *E. viscosus* DC., *E. humilis* Bieb. and *E. elatus* Bunge.

sphaerocephalus L., are used in East-Russia and Siberia as diaphoretica and diuretica, are also applied in skin diseases. In olden times, the "Herba echinopsidis" was also used in Europe for treating gravel and stone. To Dr. G. VAN VLOTEN of Leiden I am indebted for a note on the use of this genus by the Arabs. IBN WAHSCHIJA states in his treatise: *De Venenis* (cod. Leiden) the following particulars about a plant which he calls "Djirdàma":

"Djirdama grows at Djukha and at Schafiatha (in Babylonia), and is a powerful poison which kills quickly. It is a tall plant with small leaves, its stem attains the height of a meter. It has a white roselike flower and its taste is even more pungent than that of mustard. A person who has had 2—2,5 drachms of the pulverized plant administred in his food feels a violent itching on the surface of his body and a twisting and pains in the throat and the stomach and a violent burning, so that he often undresses and sits down naked. A weight of two "daniq's" administred in a beverage to pregnant women causes abortion, and a little of the powder rubbed on the skin causes burning and inflammation."

It is questionable whether this plant is really meant for an *Echinops*, as the description corresponds more with that of a pungent crucifer. The name, however, agrees with that of FORSKAHL, *Flora aegyptiaco-arabica*; but it must not be forgotten that FORSKAHL's names are of modern times, whilst those of IBN WAHSCHIJA date from about 800—900. It is, however, true that *Echinops* has indeed been proved to contain a rapidly killing poison, while if the last line of IBN WAHSCHIJA is intended for the pappus, this is also in complete accordance with the facts that it burns on the skin exactly like itchpowder. (*Mucuna.*)

A notice in the *Pharmacographia Indica* seems important, that *Echinops echinatus* DC. is an Indian medicinal plant, called in sanskrit "Utáti" and sold in the bazaars as "Utkátara". The root is bitter and serves as a tonic and diuretic. I may not, however, omit to state that Prof. Dr. H. KERN of Leiden does not believe Utáti to be a sanskrit word and said that *Echinops* is not to be found in literature on ancient Indian medicine. Messrs. D. HOOPER and G. WATT of Calcutta, coeditors of the said *Pharmacographia*, could not as yet give me further particulars on the subject of Utáti, but they have promised to order material of this drug for me from Mysore, to ascertain whether the action is due to echinopsine.

For the preparation of Echinopsine chiefly use has been made of the above mentioned fruits of *Echinops Ritro* L., collected for this purpose by Mess^{rs}. HAAGE and SCHMIDT of Erfurt. The first difficulty experienced with this material was its unusual bulk, which excluded the use of extraction-apparatus of ordinary size. Fully two-thirds of it was a straw-like chaff, a stiff tile-like involucrum, which could only be separated with very great difficulty from the fruit proper. A great deal of trouble was caused by the sharp hairs on the fruit, acting on the skin like itchpowder; by rubbing the fingers with oil this could be somewhat guarded against. The fruits yield $^2/_3$ of seed and $^1/_3$ of chaff, but the commercial article consists to the extent of one-half of empty fruits. The hard exterior (yielding 5,4 pCt. of ash) does not contain alkaloid, but yields a dark colored extract which impedes the purification of the alkaloid contained in the fruits. An aqueous decoction of the fruits tastes bitter yet at 1 : 3000—4000, but that of the involucrum is tasteless. It is, therefore, advisable to remove the involucra in order to obtain a cleaner and less bulky material, but this end cannot be attained either by crushing and grinding, or by sifting; the only way is by peeling the fruits by hand, but this is very tedious work. Under those circumstances, I have called in the aid of the governor of the penitentiary at Haarlem where this labour of separating the chaff has been performed by convicts. One kilogram crude material contains 36000 fruits and measures 10 dM^3.

The purified material (32 pCt. by weight of the original) was passed through a sieve, to remove the hairs, ground next and again sifted to retain pieces of the fruit-shell. The powdered seed boiled with 10 times its weight of alcohol of 95 pCt. yielded at the first extraction 19,2 and at the second 4 pCt. of extract, total 23,2 pCt. which high percentage is caused by the fatty oil from the seeds which has dissolved in the alcohol. The material was, therefore, first deprived of its oil by extraction with below 50° rectified petroleum ether, which does not dissolve any alkaloid. The powder may also be moistened with an equal weight of ether and then strongly pressed; nearly all the oil is thus removed with the ether. This seedcake was then dried, again pulverized and now percolated to exhaustion with alcohol of 95 pCt. mixed with 3 pCt. of acetic acid. A good yield is also obtained by boiling a few times with alcohol containing acetic acid and pressing warm each time. Of the straw-yellow tincture the alcohol was distilled off. The remnants of this extraction were only bitter at 1 : 150, being $^1/_{20}$ to $^1/_{27}$ of the original bitterness. The alcoholic extract had a peculiar ozonelike odour; it was taken up with water and filtered; remained on the filter a little of a not-bitter resin, but the filtrate was intensely bitter. This was once more shaken out with petroleum ether, a large quantity of chloroform added, the acid nearly neutralized with sodium carbonate and the whole thoroughly shaken, after the addition of an aqueous solution of caustic potash, slightly in excess. The extraction with chloroform was repeated three times; all the alkaloid goes into the solvents; after distilling off the chloroform, it remains as a light-yellow crystalline mass which dissolves readily in alcohol; the solution is strongly green fluorescent. This solution is decolorized by animal charcoal, but it retains its fluorescence, which property is shared by the crystals. There is, however, a liquid extremely well adapted to complete the purification of the crystalline vegetable base present in this complex; it is pure benzene. This readily dissolves the alkaloid by warming, but on cooling off separates practically all out, leaving the fluorescent admixture in solution.

In this manner, by repeated crystallisation until a substance of constant melting point is obtained and also by the judicious use of animal charcoal, a pure and unmixed chemical body is obtained, Echinopsine. This substance may also be obtained in an equally pure state by a repeated crystallisation from boiling water.

In this way 0,5 pCt. of Echinopsine, was obtained from the chaff-deprived fruits of *E. Ritro*; about equally large is the yield of Echinopsine, from the fruits of other species, analysed by me, such as *E. bannaticus*, *exaltatus*, *globifer*, *niveus*, *paniculatus*, *sphaerocephalus*, *syriacus*, *viscosus*; the yield of Echinopsine from select material of *E. humilis* and *elongatus* was considerably higher; from the first named species it amounted to 1,20. pCt. (!), the other yielded 0,84 pCt. Material received from Erfurt in February 1900 also yielded quite 0,8 pCt. of Echinopsine and in addition 0,1 pCt. of Echinops-fluorescine and 0,15 pCt. of Echinopseine. The amount of alkaloid in the leaves of *E. bannaticus*, *dahuricus*, *nivalis*, *platylepis*, which like those of *E. Ritro* hardly taste bitter, does not exceed 0,01 pCt. in the fresh or 0,04 pCt. in the dry material. It is considerably higher in the leaves of *E. microcephalus*, *viscosus* and *globifer*, which are all perceptibly bitter. From the fresh roots of *E. Ritro* about 0,1 pCt. of Echinopsine may be prepared.

Echinopsine obtained by this process crystallises in thin colourless needles of several cM. in length, forming feathery groups. As has already been shown, it possesses the general properties of an alkaloid; it contains nitrogen, and leaves no ash. It is a weak base; the crystals when pressed between moist red litmus paper do not colour this blue. The melting point is exactly 152° C. When heated higher Echinopsine remains unaltered for a long time, then decomposes and burns with a sooty flame.

Echinopsine dissolves 1 : 60 in water at 15°; in boiling water it dissolves very readily 1 : 6. The alkaloid practically all separates from the saturated solution on cooling, first anhydrous; the fluid then solidifies to a snow-white mass liquifying again upon the addition of hydrochloric acid. Echinopsine dissolved in water shows very beautifully the phenomenon of supersaturation; the introduction of a minute crystal into the solution soon causes an abundant separation of the alkaloid. On slow evaporation, Echinopsine may be obtained in large transparent hydrated crystals; these become opaque when heated with water, before dissolving, owing to loss of water of crystallisation.

Echinopsine is easily soluble in methyl-, ethyl- and amyl-alcohol, not so easily in carbon disulphide, insoluble in petroleum ether. The base is soluble in ethyl ether when freshly precipitated, but when crystallised it requires about 600 parts of that solvent at 15°; this is the reason why ethyl ether is not suitable as an extraction liquid for Echinopsine. Chloroform is a very suitable fluid, which dissolves the alkaloid at the ordinary temperature in all proportions and leaves it in a unaltered state on evaporation. Benzene dissolves it but sparingly in the cold (15°), but easily at 80°, about 1 in 10; this fluid is, therefore, well adapted for the purification of Echinopsine. The hydrated base is soluble in benzene with much more difficulty than the anhydrous compound; addition of water to the cold solution of the latter therefore causes a further separation of alkaloid.

The solutions of Echinopsine are all colourless and do not show fluorescence, neither when acidified with sulphuric acid.

Echinopsine is optically inactive (a 2,5 pCt. alcoholic solution examined in a 10 cM. tube showed no polarisation at 15°.)

An aqueous solution of echinopsine faintly acidified with hydrochloric acid is a bitter-tasting liquid; a hypodermatical injection of 10 milligrams in a mouse proved fatal. Prof. Dr. R. KOBERT of Rostock has, at my request, closely studied the poisonous action (see Addendum I).

Echinopsine gives precipitates with phospho-molybdic acid, solution of iodine, Mayer's reagent, picric acid, tannin, mercuric chloride, gold- and platinic chloride, potassium thiocyanate, potassium ferrocyanide and potassium chromate. The delicacy of these general alkaloid-reagents is but moderately great; one drop of a solution of echinopsine $^1/_{1000}$ gives precipitates with a drop of all the said reagents; solutions of $^1/_{10000}$ only with the first five, of $^1/_{100000}$ only with the first two. Solutions of $^1/_{25000}$—$^1/_{30000}$ are to me hardly bitter; this is also the limit of the picric acid and mercuric-potassium iodide test. (Mayer's reagent).

The latter reagents are well adapted for micro-chemical reactions but an aqueous or alcoholic solution of iodine is so in a still higher degree (limit 1 : 100000); the crystalline precipitates obtained with mercuric chloride, potassium thiocyanate, potassium ferrocyanide and potassium chromate are also very useful.

The localisation of Echinopsine in the tissues may be very plainly traced by the aid of iodine solution which yields a beautiful *crystalline* precipitate in the cell. This study has been undertaken by Prof. Dr. Ed. Verschaffelt at Amsterdam, who will communicate his preliminary results in Addendum II.

Both the anhydrous and hydrated Echinopsine excel by crystallising unusually easily; from every solvent even traces of alkaloid leave a beautiful crystalline spot. The hydrated crystals belong to the rhombic system.

Echinopsine, although a weak base, is very stable.

Echinopsine does not decompose, when melted, until 350°, when it gradually chars, but even after having been heated for an hour at 450°, the liquified mass yield yet about one-third of unaltered alkaloid. Melted with potassium hydroxide it gradually forms a redlead-coloured resin, whilst ammonia is being evolved and an odour of pyridine is perceptable. Echinopsine dissolves almost colourless in mineral acids, also in sulphuric acid on adding weak or strong oxidising agents. It also yields, under circumstances to be investigated later on, particularly by the action of acids by a high temperature, a decomposition product, which may be recrystallised from water and then appears as brown hard nitrogenous crystals which still give alkaloidal reactions, may be extracted from an acid fluid, by means of chloroform, and melt at 198°.

Echinopsine has a special reaction which should not be overlooked. Moistened with a dilute solution of ferric chloride it gives a fine blood-red colour; other colour reactions have not yet been observed.

This base forms a number of salts eminently crystalline but of a loose combination; the amount of water of crystallisation is not constant.

The first combustions of the Echinops-alkaloid did not give concurring figures for carbon. The melting point was not only raised, (at first it was 140°), when the total alkaloid, however colourless, was still further purified, but the percentage of carbon (at first 73 pCt.) [1]) increased owing to the previous admixture of accompanying alkaloid closely related to Echinopsine. But even the analysis of chemically pure Echinopsine presents difficulties; this substance is extraordinarily troublesome to ignite and gives easily a too low carbon figure unless it is ignited in a current of oxygen. I will

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[1]) *Analyses of the total alkaloid:*

0,1760 gr. gave 0,4734 gr. of CO_2 and 0,0950 gr. of H_2O, therefore C. 73,4 pCt. and H 6,0 pCt.
0,1366 " " 0,3650 " " " 0,0818 " " " " 72,9 " " " 6,6 "
0,1522 " " 12,3 c.c. of N. at 13° and 765 m m. therefore N. 9,5 pCt.

only mention here those elementary analyses, which have been used as the base of the formula. A part of the analyses was done by Mr. J. **SACK**, assistant in this laboratory.

Estimation of carbon and hydrogen.

I.	0,1758	gr. of Echinopsine gave	0,0950	gr. of H_2O and	. . . .	gr. CO_2
II.	0,1522	〃 〃 〃	0,0844	〃 〃 〃	0,4290	〃 〃
III.	0,2208	〃 〃 〃	0,1194	〃 〃 〃	0,6186	〃 〃
IV.	0,1196	〃 〃 〃	0,0606	〃 〃 〃	0,3368	〃 〃

therefore:

	I.	II.	III.	IV.
H.	6,0 pCt.	6,2 pCt.	6,0 pCt.	5,6 pCt.
C.	———	76,9 〃	76,4 〃	76,8 〃

Estimation of nitrogen.

0,2100 gr. of echinopsine analysed by the Kjeldahl-method consumed 11,6 cc. of N./$_{10}$ sulphuric acid, corresponding with 7,7 pCt. of nitrogen.

0,2410 gr. consumed 12,8 cc. N./$_{10}$ acid, corresponding with 7,4 pCt. of nitrogen.

Determination of the molecular weight.

						Mol. Weight.
0,0820	gr. of echinopsine in	17,5	gr. of benzene	gives an increase of	0,07°	157
0,5063	〃 〃 〃 〃	11,9	〃 〃 alcohol	〃 〃 〃 〃	0,28°	175
0,5740	〃 〃 〃 〃	17,5	〃 〃 benzene	〃 〃 〃 〃	0,46°	185
0,8310	〃 〃 〃 〃	17,5	〃 〃 〃	〃 〃 〃 〃	0,70°	177
0,9890	〃 〃 〃 〃	17,5	〃 〃 〃	〃 〃 〃 〃	0,79°	186
0,1990	〃 〃 〃 〃	17,5	〃 〃 〃	〃 〃 〃 〃	0,16°	185
0,5020	〃 〃 〃 〃	17,5	〃 〃 〃	〃 〃 〃 〃	0,43°	174

The elementary composition may be expressed by the formula $C_{11} H_9 NO$. The analytical figures also agree well with $(C_{11} H_{10} NO)_2$, but this formula must be rejected on account of the results of the determination of the molecular weight.

	Found.						Calculated for $C_{11} H_9 NO$.
	I.	II.	III.	IV.	V.	VI.	
H.	6,0	6,2	6,0	5,6	—	—	5,3
C.	—	76,9	76,4	76,8	—	—	77,2
N.					7,4	7,7	8,2
O.	—	—	—	—	—	—	

The calculated molecular weight of this formula is 171; the average of the found molecular weight is 177.

Estimation of water in hydrated echinopsine.

Found.				Calculated for $C_{11} H_9 NO$, aq.
10,3 pCt.	10,0 pCt.	10,0 pCt.	9,3 pCt.	9,5 pCt.

Analysis of some salts of echinopsine.

Echinopsine hydrochloride. Is a gritty crystalline powder, easily soluble in warm water, and even in the cold more freely soluble than the free base. If a crystal of hydrated Echinopsine is added to a drop of dilute hydrochloric acid, it changes into a white crystalline powder, which disappears on warming. On slow evaporation the salt is deposited in fine, large rhombohedra, on rapid evaporation in microscopic six-sided plates. The hydrochloride is well adapted for physiological experiments; at first it tastes acid, afterwards persistently bitter. It loses hydrochloric acid already at 105°. The air-dried salt, pressed between blotting-paper, retains from 6,9—14,4 pCt. of water (2 mols. of water = 14,8 pCt.), which it soon loses when placed in a dessiccator over sulphuric acid.

Amount of hydrochloric acid (of the anhydrous salt).

1) 0,2080 gr. takes 0,972 cc. N./potash or 0,0352 gr. or 16.9 pct. of H Cl.
2) 0,1367 „ „ 0,631 „ „ „ 0,0232 „ „ 16.9 „ „ „
3) 0,2147 „ „ 1,025 „ „ „ 0,0374 „ „ 17.4 „ „ „

Found.	Calculated for $C_{11} H_9 NO, H Cl$
16,9 pct. 16,9 pct. 17,4 pct.	17,7 pct.

Echinopsine sulphate. Crystallises very beautifully in elongated colourless needles, which dissolve slowly in cold but easily in warm water.

The sulphates prepared by me contained respectively 26,0 pct. (8 mols. = 24,6 pct) and 8,2 pct. (2 mols. = 7,6 pct.) of water.

Amount of sulphuric acid (calculated on the anhydrous sulphate).

1) 0,1777 gr. of anhydr. sulph. takes 0,840 cc. of N. potash or 0,0412 gr. or 23,2 pct. of $H_2 SO_4$
2) 0,1139 „ „ sulph. + 2 aq. „ 0,490 „ „ „ „ 0,0240 „ „ 22,9 „ „ „

Found	Calculated for $(C_{11} H_9 NO)_2, H_2 SO_4$
23,2 pct. 22,9 pct.	22,3 pct.

Echinopsine nitrate. Is also crystalline and not easily soluble in cold, easily soluble in warm water.

Amount of nitric acid (of the anhydrous salt).

1) 0,1462 gr. takes 0,640 cc. N.potash or 0,0403 gr. or 27,5 pCt. of HNO_3
2) 0,0521 „ » 0,230 „ „ „ 0,0145 » » 27,8 „ „ »

Found	Calculated for $C_{11} H_9 NO, HNO_3$
27,5 pCt. 27,8 pCt.	26,9 pCt.

Echinopsine oxalate. A beautifully crystallised salt which, when air-dried, contained 18,1 pCt. of water (4 mols. = 14,3 pCt.).

Amount of oxalic acid (in the anhydrous salt).

0,1777 gr. takes 0,830 cc. N.potash or 0,0373 gr. or 20,9 pCt. of $C_2 O_4 H_2$

Found	Calculated for $(C_{11} H_9 NO)_2, C_2 O_4 H_2$
20,9 pCt.	20,8 pCt.

Echinopsine picrate. A yellow crystalline salt very slightly soluble in water, of varying composition and melting at about 215°, decomposing hereby. The picric acid, present in the alkaloidal salt and obtained by shaking with petroleumether after decomposition with sulphuric acid, amounted to 81,1 pCt.

On combustion 0,1040 gr. of the same picrate gave 0,0380 gr. of H_2O and 0,1598 gr. of CO_2.

	Found	Calculated
C.	41,9 pCt.	40,0 pCt.
H.	4,0 „	3,6 »

Echinopsine mercuric chloride. Is beautifully crystalline and melts exactly at 204°. It dissolves easily in boiling water, but requires 120 parts of water at 15°.

Echinopsine mercuric iodide. The precipitate caused in a solution of echinopsine containing a slight excess of hydrochloric acid by Mayer's reagent is a yellowish-white, sticky, substance, which becomes coarsely crystalline when recrystallised from alcohol of 50 pCt. and melts at 178°. 0,150 gram of echinopsine yielded 0,455 gram of this bi-iodide, dried at 100°.

Found	Calculated for $(C_{11}H_9NO, HJ)_2 + HgJ_2$.
33,0 pCt. of alkaloid.	32,6 pCt.

Echinopsine acetate. Is also crystalline and readily soluble, even in cold water, to a bitter fluid; the salt is very unstable and loses its acetic acid completely at 100°.

Iodo-echinopsine. The crystalline precipitate produced by a solution of iodine in a liquid containing echinopsine differs in colour and composition according to the concentration and excess of the iodine employed; it also readily loses some of the iodine. It is somewhat soluble in boiling water and separates on cooling with a light-chocolate colour. When carefully dried, washed with carbon disulphide and recrystallised from alcohol, it forms a coffee-coloured crystalline powder, which melts at about 135°, but gets already sticky before that temperature is reached.

As regards the nature of Echinopsine, the following should be observed. It cannot escape notice that this substance behaves chemically more like an amide than an amine, namely like a cyclical amide, while the physiological action is strychnine-like similar to piperidone, pyrrolidone etc. To this may be added that the colouration with ferric chloride and the empirical composition also seem to point to a substance as phenylpyridone. I made some reduction and oxidation experiments ¹) to learn the structure of Echinopsine,

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¹) *Reduction of echinopsine.* Heated in a combustion tube with zinc dust in a slow current of hydrogen, echinopsine yields a distillate consisting of a yellowish-brown oily liquid having the odour of pyridine; it is heavier than water and insoluble therein but is readily dissolved on adding hydrochloric acid. This solution was

but the greater portion of my material had already been exhausted by the general study of this new substance. I can only say, that Echinopsine, although not identical with the phenylpyridone described in the *Berl. Ber.* XXIX, 1697 is probably related to the same.

The analysis of *Echinops* is not completed with the investigation of crystalline Echinopsine.

There are, namely, indications that other special substances occur in this material. In the first place it must be observed that the crystalline Echinopsine possesses only a part of the bitterness of the raw material; a decoction of the fruits is still bitter in the proportion of 1 : 3000—4000, Echinopsine hardly any more at 1 : 30.000; there must, therefore, exist some other active components which cause or increase this bitterness.

The precipitate from Mayer's reagent in the acidulated aqueous solution of alcoholic extract of echinops is much more considerable than can be accounted for by the quantity of echinopsine which might be prepared from it, and it even amounts to 0,2 gram for 1 gram of seed, being of a different nature than the precipitate obtained by Mayers reagent in an acidulated watery solution of pure Echinopsine; it has for instance a much higher melting-point.

I have devoted no small amount of labour to the study of these other constituents, but for the present I can only offer the Echinopsine in a pure condition and venture some information about the accompanying alkaloids, without wishing to pretend that the following alone account for the missing echinops-alkaloid-complex.

It has already been mentioned that the purified total-alkaloid, when repeatedly recrystallised from benzene, gradually acquires a higher melting-point. There is present a crystalline accompanying alkaloid

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repeatedly washed with ether, the base was liberated with aqueous caustic potash, distilled in a current of steam and removed from the milky distillate by means of ether. It was thus obtained as a colourless liquid of the same main properties as the crude distillate, namely heavier than water and insoluble in the same. With hydrochloric acid it forms a compound soluble in water of a burning taste giving crystalline precipitates with picric acid (yellowish-white), platinum and gold chlorides (first yellow, afterwards pale-red) which all melt and decompose at 200°; also a compound with mercuric chloride consisting of velvet-like white needles melting at 159°. These data do not admit of any identification with one of the known phenylpyridines.

Oxidation of echinopsine. Echinopsine was oxidised in the cold with 6 times its weight of a neutral 4 pCt. solution of potassium permanganate, the filtrate was treated with carbon dioxide, evaporated to dryness and the residue extracted with alcohol containing hydrochloric acid; this left undissolved a nitrogenous, hygroscopic substance soluble in water but insoluble in ether. It begins to melt at about 120° and yields on stronger heating an oily distillate having the odour of pyridine and diphenylamine.

(*β-Echinopsine*), which behaves in most respects like Echinopsine, but passes readily from the acid solution into chloroform, gives no colour reaction with ferric chloride, contains less carbon than Echinopsine, is a still more weak base and melts at 135°.

Mention has also already been made of the substance soluble in water, alcohol, amyl alcohol and benzene, which causes the green fluorescence of the solutions of the crude alkaloid, *Echinops-fluorescine*. The benzene motherliquor obtained in the preparation of Echinopsine, leaves on evaporation a dark brown mass; this was dissolved in dilute acetic acid, washed with petroleumether and ethylether and then again shaken out with chloroform; the fluorescine passes from the acid, but more readily from the alkaline solution, into that solvent. It was dissolved in acidulated water and precipitated with picric acid. The picrate, after being washed with water and dried between blotting paper, formed a sulphur-yellow crystalline cake melting at 210°. This picrate was decomposed with an aqueous caustic potash of 10 pCt. and the base thus liberated was taken up with chloroform; it seemed to be admixed with much Echinopsine. After this had crystallised out, the fluorescine remained as a brown resinous substance of alkaloidal nature, melting at 105°, not bitter, and with an extraordinarily large fluorescing power. The green fluorescence of the light brown solution is not changed by alkalies; addition of acids renders it colourless but on exposure to the air it soon regains its colour and fluorescence. The yield of fluorescine is small, the fruits of *E. exaltatus* containing a larger quantity of it than any other species, examined yet. To judge from the picrate precipitate, the purified material of *E. Ritro* contains about 0,10 pCt.

There is also in the motherliquor a non-fluorescent amorphous alkaloidal constituent, *Echinopseïne*, present. It is a brown mass decomposing on the waterbath and turning cherry-red thereby; this change of colour is also caused by alkalis. From an acid, but more readily from an alkaline solution, it passes into chloroform. The solution in very dilute sulphuric acid is bitter-adstringent, has a flavour of benzylaldehyde and gives with picric acid an abundant yellowish-green precipitate, also melting above 200°, decomposing thereby. This picrate was also decomposed by aqueous caustic potash and the base dissolved in chloroform; the chloroform residu, which was cherry-red, still contained much Echinopsine; the Echinopseïne being obtainable only as a resinous mass, melting at 125°; I therefore, had to give up further research in this direction.

Both Echinopseïne and Echinops-fluorescine obstinately adhere to

Echinopsine, causing this to exhibit for a long time a green fluorescence and to turn occasionally pink, when moistened with distilled water. This colourreaction is caused by a trace of alkali, presents in the distilled water, from the glass vessel.

Finally a few words on *Echinops oil*, which is met with when extracting the alkaloid. When quantitatively estimating the oil by extraction with ether, 27,5 pCt. was found in the seed of *E.Ritro*. It is a pale yellow sweet thick oil, of 0,930 sp. gr. at 15°, slowly drying. It has the striking property to dissolve on warming in an equal volume of absolute alcohol; on cooling an emulsion is formed and then the oil separates almost completely; at 15° the oil requires about 25 parts of alcohol for solution. Methylalcohol does not possess this remarkable property [1]). The oil is soluble in all proportions in kerosene, ether, carbon disulphide and benzene, also in an equal volume of warm glacial acetic acid. The saponification number of the oil is 194°, the melting point of the solid fatty acids 41° and the solidifying point 39°.

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ADDENDUM I.

On the physiological action of echinopsine.

By Professor Dr. R. KOBERT.

In October 1899, I received from Dr. M. GRESHOFF of Haarlem half a gram of crystallised Echinopsine hydrochloride.

With this small quantity only 3 experiments could be made with frogs and 2 with guinea-pigs.

These, however, sufficed to establish the following facts:

1. Echinopsine hydrochloride is a poison for cold-blooded and warm-blooded animals (frogs and guinea-pigs).
2. With both classes of animals the actions are similar and consist of an irritation of the motor-centres of the nervous system.
3. Both brain and spinal chord are taking part in this irritation. The irritation of the brain is only noticed in the case of warm-blooded animals and then shows itself as trismus and most violent spasmodic contraction of the masseteres. The irritation of

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[1]) Other fatty oils from the seeds of the Compositae are also more soluble in boiling alcohol than is usually the case, but not to such an extent as Echinops oil. Madia oil for instance, requires 6 parts of boiling and 30 parts of cold alcohol.

the spinal chord which, in the case of the frog, is not stopped by severing the brain, is apparent from the convulsion of all the four extremities. In the case of warm-blooded animals these may appear as klonus and tonus occasionally even as opisthotonus.

4. When a very large dose is administred to a frog, the irritation instantly passes into paralysis, whilst with a smaller dose the irritation symptoms may continue for 4—5 hours.

5. When a dose is administred to cold-blooded animals in sufficient quantity to cause irritation, it will be noticed that before the first convulsions set in and during the intervals, there exists a state of torpor, dotage and reflex-debility.

6. In the experiments on frogs the heart is decidedly weakened and such by doses which do not yet paralyse the spinal chord.

7. The complete action of echinopsine reminds of that of a mixture of strychnine and brucine but is not identical with the same, as the ophisthotonus and the reflex-irritation are not so marked as with minimal doses of strychnine and also because the heart is more affected than is the case with strychnine.

8. Doses: A subcutane dose of 0,02 gr. does not affect esculentae of ordinary size (winter frogs); 0,05 gr. causes an irrition lasting, with intervals, for several hours; 0,08 gr. paralyses the nervous system without previous irritation and also paralyses the heart at the same time.

A dose of 0,10 gr. has no visible effect on a guinea-pig weighing 325 gr., but 0,25 gr. kills the animal after suffering violent spasms for several hours.

9. Antidotes for echinopsine are to be looked for among those narcotics which do not weaken the heart.

10. It is not probable that anatomical changes occur in echinopsine poisoning cases, but I will pay attention to this matter when making experiments with the fresh material recently received from Haarlem.

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ADDENDUM II.

On the localisation of echinopsine.

By Professor Dr. E. VERSCHAFFELT.

The research on the microchemical localisation of echinopsine in the tissues will form the subject of an elaborate paper in which it will also be attempted to trace the relation between this localisation and the physiological signification of the alkaloid. Provisionally, attention will only be called to a few particulars respecting the distribution of echinopsine in the fruit of *Echinops Ritro*. For this purpose the method originally proposed by **Errera** was employed [1]), which is based on the precipitation of the alkaloid in the cells by means of iodine dissolved in potassium iodide or alcohol. With some plants mistakes may be made when using this method on account of the presence of other substances which also give precipitates with iodine such as amines, glucosides, albuminoids; but when dealing with *Echinops* no fear need be entertained as the iodo-echinopsine precipitate is not like the others [2]) in the form of a minute granular brownish-red precipitate but in large exceedingly characteristic crystals. The crystals formed in the tissues will be found under the microscope to be similar in appearance to the iodo-compound of pure echinopsine. As solutions of iodine were so eminently satisfactory it was not thought necessary to use other reagents on an extensive scale. The manner these behave towards the alkaloid in the tissues will be mentioned later on.

The scales of the involucrum which surround the ripe fruit in a dry condition, are free from alkaloid just like the dry fruit walls and their toothed hairs. The cells of the embryo, on the contrary, are mostly rich in alkaloid. This fleshy straight embryo practically occupies the space of the coalesced fruit-wall and seedcoat as far as the latter is developed. The embryo is surrounded with a double layer of thick-walled cells which like the cells of the embryo itself are filled with reserve material.

The morphological nature of this membrane which easily detaches either way from the embryo, as well as from the fruit-wall, cannot be explained with certainty without watching the course of development. It may be a rudimentary endosperm, also a seed integument. The cells of the embryo contain fatty oil and albuminoids as reserve materials. The fatty oil may be rendered visible in the ordinary way by killing the cells, for instance, by heating or by means of an acid which causes the oil to be liberated and collect in large drops. The cells are closely filled with aleuron-granules, which are present in such large numbers that they are only separated from each other by a network

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[1]) **Errera**, **Maistriau** et **Claustriau**. *Ann. Soc. Belge de Microsc.* 12, 1889. **Errera** *ibid.* 13, Mémoires.

[2]) Compare the researches of **de Wèvre**, **de Wildeman**, **Anema**, **Molle**, **Lotsy**, **Barth** and others.

of thin plates consisting of amorphous oil-containing protoplasm and often flatten one another (see Fig.). These aleuron-granules are small, their diameter being at the most one third of the size of those of *Ricinus* and *Linum*, but they are fairly equal in size. Their further structure may be silently passed over.

The cells of the already mentioned double layer present around the embryo also contain granules of an albuminous nature but these are much smaller than the aleuron-granules of the embryo.

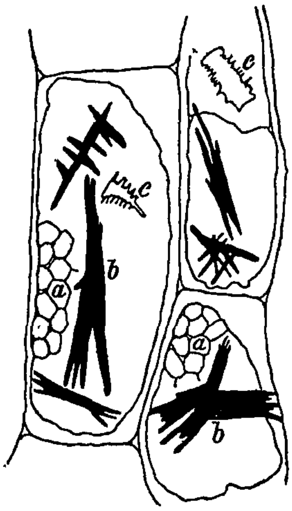

Fig.

a. borders of the aleuron-granules.
b. most numerous needle-shaped aggregations of the iodo-echinopsine compound.
c. less numerous brown plates.

Annexed figure gives a representation of a group of cells from the cotyledons of *Echinops Ritro* after treating a section with glycerol mixed with tincture of iodine until the mixture assumed a mahogany-brown colour. I have made frequent use of this mixture as well as of iodine dissolved in potassium iodide. After the sections had stayed for a while in the mixture, they were preserved and mounted in pure glycerol.

The figure does not, however, show what is seen the moment the objects are treated with the reagent, then large crystals are not formed at once. In the beginning a minute brownish-red granular precipitate is obtained which, however, unites after a few minutes to the larger aggregations of dark coloured needles, as shown. It is interesting to watch under the microscope the first formation of the precipitate; it then appears to form in the aleuron-granules which instantly turn brownish-red and show afterwards inside their mass darker and larger crystals. The amorphous protoplasm between the aleuron-granules turns at once pale yellow and remains so. Echinopsine occurs, therefore, only in the aleuron-granules and was in consequence formed within the *vacuolae* of the unripe seed, which is as might be expected.

The crystals which are visible in the cells after some time belong to two very plainly different forms. The more numerous are dark coloured manifoldly-grouped needles *b*. These agree, as regards appearance, very well with the precipitate caused in a solution of pure echinopsine of which Dr. Greshoff was kind enough to present me with a certain quantity. Between these needles are noticed a smaller number of light brown, more plate-like crystals of a peculiar feathery appearance *c*, which I have not been able to observe in the iodine-precipitate of the pure alkaloid, at least under the conditions in which I worked, so that I feel inclined to suspect the presence of the iodo-compound of an accompanying alkaloid. The double peripheric layer of the seed contains alkaloid. In the cotyledons, a beginning of differentiation is observable in palisade and spongy parenchyma, a phenomenon occurring in different plants the cotyledons of which afterwards turn green and assimilate (for instance, *Brassica*, *Linum*). There is, apparently, no difference in the amount of alkaloid contained in the tissues. The epidermis of the cotyledons also contains much alkaloid. The procambium bundles which traverse the seed lobes are, on the other hand, perfectly free from alkaloid and the same is true of those of the root. The bark of the latter is quite as rich in echinopsine as the tissue of the cotyledons.

The centre of the root which is surrounded by the procambium bundles is poor in alkaloid, so that here, a cylinder poor in alkaloid is separated from the bark rich in alkaloid by a layer free from alkaloid.

This want of alkaloid in the procambium of the embryo is interesting because, as will be more fully demonstrated later on, tolerably much alkaloid is actually found in the bast (phloem) in the further course of the development.

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Physics. — "*On the relation between Radiation and Molecular Attraction*". By J. D. VAN DER WAALS JR. (Communicated by Prof. J. D. VAN DER WAALS).

At the end of a paper in the Proceedings of the Royal Acad. of Sciences of March 1900 I expressed my intention of investigating whether the ponderomotoric action of radiation could give an explanation of molecular attraction. The course which I would take, was the solution of the equations of motion of a number of vibrators which act on each other and are subjected to no other forces. If we could solve these equations, and if this action proved sufficient to explain the molecular attraction, we might be able to deduce from this whether the quantity a of the equation of state is a function of the temperature, and if so, what function, and whether the attraction is really proportional to the square of the density, or if it is so only by approximation.

I have however, not succeeded in finding the function solution of this problem, not even for the case that there are only two