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THE ALKALOIDS OF ERGOT

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XXXVII.—*The Alkaloids of Ergot.*

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The great medicinal importance of ergot has led to numerous chemical researches, which have mostly, however, resulted in little that is definite, possibly owing to the fungoid nature of the plant. As in the case of many drugs, the therapeutic properties of ergot have been attributed to the presence of one or more alkaloids, but several investigators have of late years denied to the ergot alkaloids any part in the specific activity of the drug. The problem is further complicated by the extremely small alkaloidal content (about 0·1 per cent.); this makes the preparation of even a moderate supply of material very expensive.

The first to establish the presence of fixed alkaloids in ergot was Wenzell (*Amer. J. Pharm.*, 1864, **36**, 193), who in 1864 applied the names ecboline and ergotine to two impure resinous preparations giving alkaloidal reactions. His observations were confirmed by Ganser (*Arch. Pharm.*, 1870, **194**, 195). To Tanret, however, belongs the credit of having first obtained from ergot a well-defined crystalline alkaloid, which he named ergotinine in order to distinguish it from the resinous ergotine of Wenzell and others (*Compt. rend.*, 1875, **81**, 896; 1878, **86**, 888; *Ann. Chim. Phys.*, 1879, [v], **17**, 493). From the mother liquors of this base Tanret obtained a further yield of alkaloid in an amorphous form. Since this amorphous alkaloid in other respects closely resembled the crystalline, he called it amorphous ergotinine. Tanret's crystalline alkaloid has been found by all subsequent observers. According to Blumberg (*Inaug. Diss. Dorpat*, 1878) it is probably identical with the picrosclerotine of Dragendorff and Podwyssozki (*Arch. expt. Path. Pharm.*, 1876, **6**, 153); the term sclerocrystalline used by Podwyssozki (*Pharm. Zeitschr. für Russland*, 1883, **22**, 396) is also merely another name for Tanret's alkaloid, and the same applies to the secaline of Jacobj (*Arch. expt. Path. Pharm.*, 1897, **39**, 104). According to Kobert (*Arch. expt. Path. Pharm.*, 1884, **18**, 316) crystalline ergotinine has but slight if any physiological activity, so that when he obtained an impure alkaloidal resin of great toxicity he proposed for it the new name cornutine. According to Tanret (*J. Pharm. Chim.*, 1885, [v], **11**, 309; 1894, [v], **30**, 229), Keller (*Schweiz. Wochenschr. Chem. Pharm.*, 1894, **32**, 121; 1896, **34**, 65), and Meulenhoff (*Ber. Nederl. Maatsch. Pharm.*, 1899, [viii], No. 1; *Ned. Tydschr. Pharm.* 1900, **12**, 225, 257), cornutine does not occur as such in ergot, but is an artificial decomposition product of ergotinine, formed by the acid used in its extraction.

In the course of a prolonged investigation of ergot we obtained the second amorphous alkaloid for the first time in a state of purity by crystallising its salts, and were thus able to distinguish it from ergotinine, which is itself crystalline but only yields amorphous salts. Moreover, the amorphous alkaloid is very soluble in alcohol, the crystalline only slightly so. As the second alkaloid is now also recognisable as a chemical individual, and as it has proved to be a substance of great physiological potency, we suggested for it the name ergotoxine in a preliminary communication to the British Association at York (*Chem. News*, 1906, **94**, 89. See also Report of Brit. Med. Assoc. Meeting, Aug. 1906, in *Brit. Med. J.*, Dec. 22nd, 1906, p. 1792). We found in ergotinine considerably more nitrogen than Tanret, and hence were unable to confirm his formula, $C_{35}H_{40}O_6N_4$. As we could not prepare any crystalline ergotinine salts we relied on determinations of the molecular weight of the free alkaloid by physical methods, which made us suggest the provisional formula, $C_{28}H_{32}O_4N_4$. Our analysis of crystalline ergotoxine salts indicated a close relationship between the two alkaloids, and later we proved this relationship by converting ergotoxine into ergotinine by boiling with acetic anhydride; we also obtained a crystalline phosphate closely resembling ergotoxine phosphate by boiling ergotinine with dilute alcoholic phosphoric acid. Soon after our preliminary note was published, there appeared an important paper by Kraft (*Arch. Pharm.*, 1906, **244**, 336), who was, in the first place, concerned with the examination of an ethereal extract of ergot. He lays stress on the following circumstances, which make the separation of the extract into its various constituents very troublesome: the presence of ergosterol and of fat, the feebly acidic nature of the acids, and the feebly basic nature of the alkaloids, the presence of phenolic hydroxyls in the latter giving them also acid properties. Although chemically rather inert, these colloidal bodies "unite among themselves and with fat to form quite stable adsorption compounds." It is, therefore, well nigh impossible to effect a quantitative separation of the alkaloidal from the acid constituents, for instance, by extraction with dilute acetic acid and with ammonia. Kraft concludes that the mere use of solvents and of fractional precipitation is worthless, and that for this reason the numerous ergot substances of Jacobj are but mixtures.

At these conclusions we had ourselves arrived. Acid substances, such as sphacelotoxin, owe their physiological activity to contamination with a powerfully active alkaloid, and we agree with Kraft in emphasising the physiological importance of the alkaloidal as opposed to the acidic constituents of ergot. Without knowledge of our work, Kraft prepared the amorphous alkaloid by fractional precipitation of the sulphate and observed its conversion into ergotinine, his method

being to boil a solution of the amorphous alkaloid in methyl alcohol. He regarded this change as due to the elimination of water, and accordingly suggested for the second alkaloid the name hydro-ergotinine. He did not, however, crystallise any of its salts, nor did he analyse either base. In a recent communication with H. H. Dale (*Arch. Pharm.*, 1906, **244**, 550), one of us expressed doubt as to the validity of Kraft's conception of the amorphous alkaloid as a hydrated ergotinine, because the results of our analyses, taken in conjunction with molecular weight determinations by physical means, appeared to contradict such a view, and led us to interpret the production of ergotinine from ergotoxine by means of acetic anhydride as an acetylation.

Further work, and especially molecular weight determinations of ergotoxine by chemical means, which appear in this case to be more suitable than physical methods, have made us adopt the formula $C_{35}H_{41}O_6N_5$ for ergotoxine. This formula is in satisfactory agreement with our analyses of several of its salts. We now suggest for ergotinine the formula $C_{35}H_{39}O_5N_5$, which requires practically the same percentage composition as $C_{28}H_{32}O_4N_4$ previously suggested, and which (except for the nitrogen) is similar to Tanret's original formula, $C_{35}H_{40}O_6N_4$.

The action of acetic anhydride consists therefore in the removal of a molecule of water, and not as was previously surmised (Barger and Dale, *loc. cit.*) in the introduction of an acetyl group. This earlier surmise is moreover disproved by the fact that no acetic acid is given off on boiling ergotinine with mineral acids.

Kraft showed that the change from the amorphous to the crystalline base is also produced by boiling methyl alcohol, an observation which we have since confirmed. On the basis of this experiment and the production of an amorphous base from ergotinine on standing with dilute acetic acid, Kraft suggested that "the amorphous alkaloid is the hydrate of the crystalline." This theory, which is supported by our own experiments on the action of acetic anhydride and of phosphoric acid, we now regard as definitely established by our analyses.

When the manuscript of this paper was almost complete, our attention was drawn to a recent communication by Tanret (*J. Pharm. Chim.*, 1906, (vi), **24**, 397) in criticism of our preliminary note. By means of a determination according to Dumas, Tanret has confirmed our value for the percentage of nitrogen in ergotinine. He brings evidence to show that our molecular weight determinations by the cryoscopic method in phenol are invalid on account of the production of a phenoxide, which he infers from measurements of the specific rotation. Tanret now suggests for ergotinine the formula $C_{35}H_{40}O_5N_5$,

and, as pointed out above, we have ourselves come to reject our original molecular weight determinations and to suggest the formula $C_{35}H_{39}O_5N_5$. Tanret's formula is impossible, since the total number of valencies in the molecule must be an even number. There must be either 39 or 41 hydrogen atoms. We have selected the former number as agreeing much the best with our analyses.

With regard to the relation between ergotoxine and ergot alkaloids other than crystalline ergotinine, there is little doubt that it is largely present in Tanret's so-called amorphous ergotinine, but as this is merely the residue left on evaporating the mother liquor from the crystalline ergotinine, it must contain a proportion of crystallisable alkaloid. This proportion is by no means negligible. As Tanret observed in one of his earlier papers (1879, p. 506), the presence of amorphous ergotinine greatly increases the solubility of the crystalline alkaloid, and in the same paper (p. 507) he attributes the variation in the specific rotation of "amorphous ergotinine" to varying amounts of crystallised ergotinine contained in it. Tanret therefore did not prepare the amorphous alkaloid in the pure state. The amorphous alkaloid is either the same chemical individual as the crystalline or it is not. In the former case it should be possible to crystallise it, and it should have the same specific rotation as the crystalline alkaloid. In the latter case it is confusing to apply the same name to different substances, and a new name must be found. That the amorphous alkaloid is a distinct, although closely related substance, we consider completely established. Tanret deduces the identity of the two alkaloids from the similarity of "their behaviour to precipitating reagents, of their fluorescence, of the mode of formation and properties of their salts, and the amount of acid with which they combine, and finally from the similarity of the characteristic colour reaction which they give with sulphuric acid." But all these points of similarity might be expected in the case of two alkaloids of high molecular weight, differing only by one molecule of water and readily passing into one another. Moreover, there are differences in the characteristics referred to by Tanret. We have found ergotoxine solutions to be distinctly more sensitive to many alkaloidal precipitants than ergotinine solutions of the same strength. As for the salts, few if any ergotinine salts have been obtained crystalline, whereas we have been able to crystallise quite a number of ergotoxine salts. Kraft (*loc. cit.*) has shown that a remarkable difference exists between the solubilities of the two sulphates, that of the amorphous alkaloid requiring 8,000 parts, and that of the crystalline only 500 parts of water for solution. We have confirmed Kraft's observations on this point. Tanret himself has pointed out the difference between the solubilities of the two alkaloids in alcohol and the difference in their specific rotations. Kraft

and we ourselves have transformed one alkaloid into the other by chemical means, and finally, our analyses show that the two alkaloids have a different percentage composition. The term amorphous ergotinine must therefore be restricted to such specimens of the crystallisable alkaloid as can, for instance, be prepared by precipitating a solution in dilute acetic acid by ammonia.

The question of the relation between ergotoxine and cornutine is somewhat more difficult. Kobert's method of preparation makes it probable that his substance contains ergotoxine. On the other hand, certain of the most striking symptoms described by Kobert as characteristic of the toxic effects of cornutine are not produced by ergotoxine. This made it impossible to adopt for our alkaloid the name cornutine which Kobert has associated, not with chemical properties, but with the production of a certain physiological picture (see also Barger and Dale, *Arch. Pharm.*, 1906, **244**, 554).

On the other hand, the hydroergotinine recently described by Kraft is undoubtedly identical with ergotoxine, previously described by ourselves. After an examination of ergotoxine salts prepared by us, Dr. Kraft has recently informed us that he shares our view as to the identity of the alkaloids. He, too, has now succeeded in crystallising ergotoxine (hydroergotinine) sulphate.

According to experiments of H. H. Dale, ergotoxine produces in doses of a few milligrams not only the characteristic reactions of ergot described by him (*J. Physiol.*, 1906, **34**, 163), but also gangrene of the cock's comb and other ergot effects described by Kobert and others to sphacelenic acid. Crystalline ergotinine, uncontaminated with ergotoxine, either does not give these effects at all, or only to a slight extent, the difference being possibly due to the difficulty of keeping ergotinine in solution in the body fluids.

Ergotinine.

This alkaloid was prepared by various methods, in the first place according to that given by Tanret, starting from an alcoholic extract of the drug. Great difficulty was experienced in extracting with ether, and in washing the ether with water, as the alkali present led to the formation of a resin soap, which made the separation into an ethereal and an aqueous layer very tedious. Meulenhoff mentions the same difficulty. For the preparation of ergotinine on a small scale, it is best to take advantage of the fact that it can be extracted by ether; this is the basis of Keller's method. He first extracted the ergot with light petroleum to remove the oil and then with ether to remove the alkaloid We agree with Kraft that there is no advantage in the preliminary extraction with light petroleum.

If the ethereal extract is freed from ether and the resulting oil mixed with light petroleum, most of the alkaloid, together with a yellow colouring matter and other substances, is precipitated (Jacobj's chrysotoxin), but a small part of the alkaloid remains in the oil and can be extracted with dilute acids. The alkaloid obtained in this manner is uncontaminated with resin and at once yields white crystals from alcohol.

The salts of the ergot alkaloids with inorganic acids are very slightly soluble in water. Hence Tanret extracted the ethereal solution with citric acid, and Kraft used tartaric acid. When working on a large scale, however, the slight solubility of the chloride and especially of the bromide may be put to account. The residue left on evaporation of the alcoholic tincture is extracted with light petroleum to remove fat and oily matter; it is then dissolved in ethyl acetate and shaken with citric acid solution. Sodium bromide or hydrobromic acid is added and the precipitated hydrobromides of the alkaloids are collected. A rough separation of ergotinine from ergotoxine can be effected by repeated shaking of the solution of the mixed hydrobromides in dilute caustic soda with ether; in this way the ergotinine is removed first. Finally, the ergotinine is crystallised from alcohol, leaving ergotoxine and impurities in the mother liquor.

Composition of Ergotinine.

For analysis the alkaloid was recrystallised from absolute ethyl alcohol and was dried in a vacuum over sulphuric acid or in the steam oven. It is not hygroscopic.

I. 0·1511 gave 0·3793 CO_2 and 0·0851 H_2O. C = 68·47; H = 6·30.
II. 0·1174 „ 0·2966 „ „ 0·0700 „ C = 68·90; H = 6·66.
III. 0·1598 „ 0·4031 „ „ 0·0912 „ C = 68·80; H = 6·38.
IV. 0·1557 „ 0·3914 „ „ 0·0881 „ C = 68·55; H = 6·33.
V. 0·1947 „ 0·4889 „ „ 0·1160 „ C = 68·48; H = 6·66.
VI. 0·1537 „ 0·3871 „ „ 0·0904 „ C = 68·68; H = 6·58.
VII. 0·1453 „ 0·3674 „ „ 0·0871 „ C = 68·96; H = 6·70.

In the last combustion the substance was mixed with cupric oxide.

Mean C = 68·69; H = 6·52.

$C_{35}H_{39}O_5N_5$ requires C = 68·91; H = 6·45 per cent. Tanret's analysis gave C = 68·57; H = 6·79 per cent.

I. 0·1320 gave 13·1 c.c. N at 17° and 770 mm. N = 11·7.
II. 0·1331 „ 13·0 „ „ „ 17 „ 770 „ N = 11·5.
III. 0·2156 „ 22·0 „ „ „ 21 „ 767 „ N = 11·7.
IV. 0·2283 „ 22·0 „ „ „ 12·5 „ 774 „ N = 11·6.

$C_{35}H_{39}O_5N_5$ requires N = 11·5 per cent.

Tanret found in 1879 by Will and Varrentrapp's method 8·71 and

9·26 per cent. of nitrogen, and quite recently by Dumas' method 11·76 per cent., so that the former method is once more shown to be unsatisfactory.*

Molecular Weight Determinations.

I. By the cryoscopic method in acetic acid:

0·210 in 12·85 acetic acid gave $\Delta t = -0·100°$; M.W. = 634.

$C_{35}H_{39}O_5N_5$ requires M.W. = 609.

The suitability of acetic acid as a solvent for an alkaloid we infer from an experiment with strychnine, which gave a good value (342, calc. 334) for the molecular weight.

II. By the analysis of the platinichloride, prepared by dissolving ergotinine in acetone, adding hydrochloric acid and an aqueous solution of platinic chloride, evaporating the acetone in a vacuum desiccator, filtering off the precipitated salt from the residual water and washing with water.

0·1740 of the salt left on ignition 0·220 Pt; Pt = 11·6.

$(C_{35}H_{39}O_5N_5)_2,H_2PtCl_6$ requires Pt = 11·9 per cent.

$(C_{35}H_{41}O_6N_5)_2,H_2PtCl_6$,, Pt = 11·6 ,,

The second formula is applicable if the ergotinine is changed to ergotoxine by the acid present.

The formula $C_{35}H_{39}O_5N_5$ agrees with Tanret's old analyses of the amorphous hydrochloride and hydrobromide of ergotinine, and with his recent analyses of the likewise amorphous platinichloride (found: Pt = 11·66, 11·83; Cl = 12·69; calculated Pt = 11·95; Cl = 13·06). We were first led to adopt this formula by analyses of crystalline ergotoxine salts, which gave us the corresponding formula $C_{35}H_{41}O_6N_5$ for ergotoxine. Before this time we preferred the formula $C_{28}H_{32}O_4N_4 = 488$, based on the following molecular weight determinations in which the ergotinine apparently underwent decomposition.

I. By the cryoscopic method in phenol:

0·1775 in 14·57 phenol gave $\Delta t = 0·32°$; M.W. = 516.

0·3715 ,, ,, ,, ,, $\Delta t = 0·535°$; M.W. = 477.

* At first we thought that the discrepancy with Tanret's original figures might be due to the presence of methane in our nitrogen (compare Dunstan and Carr, Proc., 1896, **12**, 48; and Haas, Trans., 1906, **89**, 570). We therefore availed ourselves of the kindness of Dr. P. Haas, who has recently investigated this source of error (*loc. cit.*). He performed the first two of the determinations quoted, mixing the substance with cuprous chloride and proving the absence of methane in the nitrogen by explosion with oxygen. For his help in this matter we tender him our best thanks. In subsequent determinations of the nitrogen in ergotinine and in ergotoxine salts we always mixed the substance with cuprous chloride. Without this precaution, too much nitrogen was found; for example, 12·0 per cent. in ergotinine.

II. By a microscopic method, due to one of us (Trans., 1905, **87**, 1756), 0·0858 in 1·06 pyridine at 80° = 0·17 – 0·18 mole.; M.W. = 463.

The number of carbon, oxygen, and nitrogen atoms in ergotinine seems now to be established with tolerable certainty. The number of hydrogen atoms is less certain; it must, however, be an odd number, in order to make the total number of valencies even, and can therefore not be forty, as Tanret suggests. From our analyses we conclude that there are very probably 39, possibly 41 hydrogen atoms in ergotinine. The formula $C_{35}H_{39}O_5N_5$ requires 6·45 per cent., the formula $C_{35}H_{41}O_5N_5$ 6·76 per cent., of hydrogen: on the average we found 6·52 per cent. in good agreement with the first formula, since the amount of water found in combustions is generally somewhat too high.

Properties of Ergotinine.

Ergotinine crystals consist of long needles, the sides of which are not quite parallel; the ends are symmetrically replaced by a pair of faces, and the extinction is straight.*

When placed in a bath at 210° and heated further ergotinine sinters, darkens and melts at temperatures up to 229° (corr.). Kraft gives m. p. 219°, Tanret 205°. We have also frequently found 219—220° (uncorr.). The decomposition point is not very characteristic and depends greatly on the rate of heating.

Solubility determinations were made by shaking the powdered alkaloid in the cold with the solvent, and by rapidly filtering the boiling solution. One part of ergotinine dissolves at 10° in 312 parts by weight of absolute ethyl alcohol, at 18° in 292 parts of alcohol, in 1,020 parts of absolute ether, in 91 parts of ethyl acetate, and in 26 parts of acetone; further, in 77 parts of boiling benzene, 52 parts of boiling ethyl alcohol, and 56 parts of boiling methyl alcohol. It is extremely soluble in cold chloroform, moderately so in amyl alcohol, methylal or xylene, and insoluble in light petroleum.

The determination of the specific rotation at 10° in a saturated solution in ethyl alcohol, prepared by shaking in the cold, gave $\alpha_D + 1·91°$; $l = 0·22$ dcm.; $c = 0·257$; $[\alpha]_D + 338°$.

Tanret found +334° and +336°. Five different specimens of ergotinine, in alcoholic solutions prepared by boiling, with $c = 0·172$—0·257, gave at 10—18° $[\alpha]_D$ +320°, +328°, +326°, +330°, +327°; mean +328°.

The rotatory power of an alcoholic solution of ergotinine falls

* For this description of ergotinine crystals and for others of the crystals of ergotoxine salts given later, we are indebted to Prof. W. J. Pope and tender him our best thanks for his assistance in this respect.

through prolonged boiling. The solution saturated in the cold, and referred to above, was boiled under a reflux condenser. $[\alpha]_D$, originally $+338°$, fell after five minutes to $+327°$, after one hour to $+300°$, after three hours' boiling to $+242°$. In another experiment a specimen of ergotinine, prepared from ergotoxine by acetic anhydride, had $[\alpha]_D$ $+388°$, but after it had been boiled for half an hour the same solution gave $[\alpha]_D$ $+326°$. The fall of rotatory power is accompanied by a disappearance of crystallisable alkaloid. We have frequently noticed a certain deficit when recrystallising a given quantity of pure ergotinine, however carefully the successive mother liquors were concentrated.

The destructive action of hot alcohol is also shown by an experiment in which 0·1 gram of ergotinine was heated with 3 c.c. of alcohol in a sealed tube at 100° for twelve hours. More than half was destroyed. Tanret, in his recent publication, has also pointed out that a solution of the crystalline alkaloid always leaves a partially amorphous residue on evaporation. We further determined the specific rotation in other solvents and found higher values than in alcohol.

In acetone: α_D $+0·86°$; $l=1$ dcm.; $c=0·234$; $[\alpha]_D$ $+367°$.
In ethyl acetate: α_D $+0·64°$; $l=1$ dcm.; $c=0·176$; $[\alpha]_D$ $+363°$.
In chloroform: α_D $+2·03°$; $l=1$ dcm.; $c=0·514$; $[\alpha]_D$ $+396°$.

The lowering of the specific rotation of ergotinine, produced, as Tanret showed, by the addition of acids and alkalis to the solution, seems to depend in the first place on a transformation to ergotoxine and possibly also on racemisation. For instance, the addition of one molecular equivalent of phosphoric acid to an alcoholic ergotinine solution lowered $[\alpha]_D$ from $+328°$ to $+319°$, and after boiling for fifteen minutes to $+195°$. After boiling with fifteen molecular proportions of phosphoric acid the value was $+41°$. Under similar conditions sulphuric acid produced a much more rapid lowering of the rotatory power, which presumably depends on the concentration of hydrogen ions.

So far we have not been able to prepare any undoubted ergotinine salts in the crystalline state. Even when crystals are obtained, there is the possibility of the formation of the corresponding ergotoxine salt as a result of hydrolytic action of the acid used. In an attempt to crystallise ergotinine phosphate we added the calculated quantity of phosphoric acid to an alcoholic solution of the base, and concentrated the solution in a vacuum desiccator. The salt which separated out was gelatinous and was filtered off; on decomposition with ammonia it readily yielded the characteristic prisms of ergotinine. The filtrate from the amorphous salt was concentrated further, and finally yielded

a small quantity of sphærites and diamond-shaped plates. These were decomposed with ammonia in the same way as the amorphous salt, but yielded an amorphous base, which withstood all attempts at crystallisation and was very readily soluble in alcohol. Later, it was found that a good yield of the same crystals could be produced by boiling the solution for a short time, and that they probably represent ergotoxine phosphate.

It is therefore probable that the crystalline sulphate mentioned by Tanret, and possibly also the halides which he analysed, together with the crystalline chloride obtained by Keller, were salts of ergotoxine. As we have shown, ergotinine is readily decomposed by very dilute phosphoric acid in boiling alcoholic solution, and, apparently, transformed to ergotoxine phosphate; Kraft has found that ergotoxine is even produced in dilute acetic acid solution in the cold. We have also obtained from ergotinine small quantities of a crystalline picrate, but are unable to say whether it is a salt of ergotinine or ergotoxine.

Keller found that the amorphous hydrochloride formed by precipitating an ethereal solution of the base with hydrochloric acid is soluble in water, but gives a precipitate with hydrochloric acid. This he attributed to the formation of an insoluble acid salt. We are unable to share this view. The salt is not only precipitated by hydrochloric acid, but also by sodium chloride, sodium acetate, and sodium tartrate; not, however, by acetic acid, tartaric acid, carbamide, or sucrose. The precipitation therefore depends on the presence of ions and the solution of the salt is a colloidal one. Ergotoxine phosphate also gives a colloidal solution (see later). It is probable that the reason why organic acids are more suitable for the extraction of ergotinine from an ethereal solution than inorganic acids, depends on the small degree of ionisation of organic acids.

The characteristic colour reactions for ergotinine have been described by Tanret (addition of concentrated sulphuric acid to a solution of ergotinine in ether or in ethyl acetate, giving a transitory orange coloration, changing to blue) and by Keller (anhydrous ferric chloride, added to ergotinine in concentrated sulphuric acid, changes the colour from pale yellow, through orange, crimson, and green to a permanent dark blue). Both these reactions are, however, given by ergotoxine with the same intensity as by ergotinine, since the two alkaloids naturally undergo the same decomposition by sulphuric acid. The behaviour of ergotinine solutions towards alkaloidal precipitants will be discussed later and compared with that of ergotoxine solutions. For a reply to Tanret's recent criticism of our view, that ergotinine is physiologically inert, we must for the present refer to the paper by Barger and Dale (*loc. cit.*).

Ergotoxine.

This alkaloid was prepared as follows. The caustic liquor from which the ergotinine had been extracted (see above, under preparation of ergotinine) was neutralised, again rendered alkaline with sodium carbonate, and extracted with ether. The residue left after evaporation of the ether, together with that from the ergotinine mother liquors, was dissolved in 80 per cent. alcohol and a slight excess of phosphoric acid in alcohol added. After standing for a few days the ergotoxine phosphate crystallised out and was recrystallised from alcohol. In the purest form in which we have obtained this alkaloid it formed a light white powder, which when heated began to soften at about 155° and gradually melted at 162—164°. It is more soluble in organic solvents than ergotinine, notably in cold alcohol. In ether it is but slightly soluble. All attempts to crystallise it, for instance, by the slow evaporation of its ethereal solution in a desiccator, have failed.

For analysis, the alkaloid was liberated from the pure oxalate with sodium carbonate and dissolved in ether. After careful washing, the solution was evaporated in a vacuum desiccator and the residual alkaloid dried until of constant weight.

0·2553 gave 0·6224 CO_2 and 0·1503 H_2O. C = 66·49; H = 6·59.
0·1982 ,, 0·4808 CO_2 ,, 0·1164 H_2O. C = 66·16; H = 6·58.
$C_{35}H_{41}O_6N_5$ requires C = 66·93; H = 6·59 per cent.

For the establishment of this formula we mainly rely, however, on our analysis of the crystalline salts.

For the specific rotation of ergotoxine in alcoholic solution we have obtained the following figures:—

I. Prepared from the oxalate by ammonia and ether,

$$\alpha_D + 0·6°;\ l = 1 \text{ dcm.};\ c = 1·624;\ [\alpha]_D + 40·6°.$$

II. By the addition of the theoretical quantity of caustic soda to the phosphate dissolved in alcohol,

$$\alpha_D + 0·31°;\ l = 1 \text{ dcm.};\ c = 0·80;\ [\alpha]_D + 44·5°.$$

III. Prepared from the oxalate by sodium carbonate and ether,

$$\alpha_D + 0·61°;\ l = 1 \text{ dcm.};\ c = 1·37;\ [\alpha]_D + 45·3°.$$

IV. Prepared from the oxalate by sodium carbonate and ether,

$$\alpha_D + 0·10°;\ l = 1 \text{ dcm.};\ c = 0·45 \text{ m.};\ [\alpha]_D + 22·2°.$$

V. Prepared from the oxalate by ammonia and ether,

$$\alpha_D + 0·005°;\ l = 1 \text{ dcm.};\ c = 0·884;\ [\alpha]_D + 0·6°.$$

All these values are very much lower than those given by Tanret for "amorphous ergotinine," which must have contained a considerable proportion of crystallisable alkaloid. At the same time we are unable to explain the want of constancy in our values unless this is

due to a varying degree of racemisation; this point requires further investigation.

We have studied the action of alkaloidal precipitants on ergotoxine and on ergotinine in some detail in order to bring out the points of difference between the two alkaloids. The following table gives approximately the most dilute solutions of each alkaloid in which the various reagents still produced a faint opalescence. The solutions were made by dissolving ergotoxine phosphate and ergotinine in the minimum quantity of glacial acetic acid and diluting with distilled water:

Reagent.	One part of ergotinine in	One part of ergotoxine in
Potassium mercuric iodide	1,000,000	2,000,000
Iodine in potassium iodide	200,000	1,000,000
Picric acid	50,000	50,000
Phosphomolybdic acid	40,000	80,000
Phosphotungstic acid..... ...	40,000	40,000
Auric chloride.....	20,000	20,000
Potassium chromate	20,000	20,000
,, dichromate	10,000	20,000
,, ferrocyanide	10,000	10,000
,, ferricyanide	10,000	10,000
,, sulphocyanate	10,000	20,000
Platinic chloride.....	10,000	10,000
Bromine water	10,000	20,000
Tannic acid	8,000	20,000
Potassium fluoride	50,000	50,000
,, iodide	20,000	20,000
,, bromide.....	6,000	15,000
Sodium chloride	4,000	8,000
,, sulphate.....	500	7,000

It will be seen that an ergotinine solution is distinctly less sensitive to a number of reagents than an ergotoxine solution of the same strength. The best reagent is potassium mercuric iodide, which, according to Tanret, precipitates a solution of 1 part ergotinine in 1,240,000 parts of water. The limits for the last three salts depend largely on the amount of salt added and are somewhat arbitrary.

Ergotoxine Salts.

These salts were mostly prepared by adding a concentrated alcoholic solution of the acid drop by drop to a dilute ethereal solution of the base obtained from the phosphate. The reaction is best carried out in a tall stoppered cylinder, so that after shaking the salt readily settles down and leaves a clear, supernatant solution. As soon as no further precipitate is formed, the salt is washed a few times with dry ether by decantation, collected at the pump, and dried in a vacuum. In the case of mineral acids, it is very important to avoid excess, which brings about decomposition.

A sulphate, nitrate, hydrochloride, hydrobromide, two oxalates, and a tartrate, have been obtained crystalline, but only the phosphate, the oxalates, and the hydrochloride have so far been studied. The salts of ergotoxine with inorganic acids are very sparingly soluble in water, but readily so in hot alcohol, from which they can be crystallised. The salts with organic acids are more soluble in alcohol and are best crystallised by adding ether to their solutions in cold alcohol.

Ergotoxine is, like ergotinine, a very feeble base and does not combine with more than one equivalent of acid. Its salts have an acid reaction; a crystal of the oxalate, for instance, produces a red spot when placed on moist blue litmus paper.

Ergotoxine Phosphate, $C_{35}H_{41}O_6N_5,H_3PO_4,H_2O$.

This is the most easily purified of the ergotoxine salts which we have so far examined, and was the starting-point in the preparation of the other salts. The crude phosphate obtained in the manner described under ergotoxine is decomposed by sodium carbonate, yielding the base, and this is precipitated in ethereal solution with phosphoric acid. After washing, the precipitated salt is dried and crystallised from alcohol, using 50 c.c. of 90 per cent. boiling alcohol for 1 gram of salt. With these proportions crystallisation should begin slowly, after one or two hours. It is important to use alcohol containing a little water, as the phosphate is much less soluble in absolute alcohol. When the crude salt is crystallised it separates in groups of needles radiating from centres and showing straight extinction, and, when pure, in isolated needles melting with decomposition at 186—187° (the bath being heated to 180° before the introduction of the substance).

One part of ergotoxine phosphate dissolves in 313 parts of cold, and in 14 parts of boiling alcohol of 90 per cent.

By shaking ergotoxine phosphate with cold distilled water, a typical colloidal solution can be obtained, containing 1 per cent. of the salt. This solution froths and is strongly opalescent, but does not deposit any of the salt on standing. The addition of an electrolyte (sodium acetate, sodium phosphate) converts the hydrosol into a gel. If equal volumes of *N*-hydrochloric, oxalic, phosphoric, or acetic acids are added to the solution, the degree of precipitation is in the order named, that is, in that of the conductivities of the acids. The hydrochloric acid produces a thick jelly, so that the test tube can be inverted, while the acetic acid leaves the solution fluid. It seems probable that this is one of the reasons why phosphoric and most organic acids are to be preferred to the stronger mineral acids in the extraction of ergot alkaloids. It has been shown already that a colloidal solution of

ergotinine hydrochloride is precipitated by electrolytes in a similar manner.

For analysis the phosphate was recrystallised two or three times from alcohol, and dried in a vacuum over sulphuric acid till constant. The salt crystallises with one molecule of water.

0·1696 lost 0·0042 H_2O; $H_2O = 2·42$.

$C_{35}H_{41}O_5N_5,H_3PO_4,H_2O$ requires $H_2O = 2·48$ per cent.

The anhydrous salt is hygroscopic, so that the boat was always enclosed in a weighing tube.

I. 0·1254 gave 0·2650 CO_2 and 0·0713 H_2O. $C = 57·63$; $H = 6·37$.
II. 0·1654 „ 0·3491 „ „ 0·0925 „ $C = 57·56$; $H = 6·26$.
III. 0·1552 „ 0·3278 „ „ 0·0843 „ $C = 57·60$; $H = 6·08$.

$C_{35}H_{41}O_6N_5,H_3PO_4$ requires $C = 57·89$; $H = 6·11$ per cent.

In each of the above three analyses a different specimen was used.

In the following two, the substance was mixed with cupric oxide, leading to somewhat higher figures, especially for the hydrogen.

0·1698 gave 0·3634 CO_2 and 0·1020 H_2O. $C = 58·37$; $H = 6·71$.
0·0916 „ 0·1954 „ „ 0·0570 „ $C = 58·17$; $H = 6·99$.

For the determination of the nitrogen the substance was mixed with cuprous chloride. The phosphoric acid was determined by heating the salt on the water-bath with ammonia, and filtering off the alkaloid.

0·1128 gave 9·4 c.c. N at 19° and 768 mm. $N = 9·7$.
0·1787 „ 14·5 c.c. N at 14·5° and 760 mm. $N = 10·0$.
0·3688 „ 0·0588 $Mg_2P_2O_7$; $H_3PO_4 = 14·1$.

$C_{35}H_{41}O_6H_5,H_3PO_4$ requires $N = 9·7$; $H_3PO_4 = 13·5$ per cent.

Ergotoxine Hydrochloride, $C_{35}H_{41}O_6N_5,HCl$.

When prepared by precipitation of an ethereal ergotinine solution this salt forms minute diamond-shaped plates, and very thin and very long square-ended needles, showing straight extinction and melting at 205° (bath previously heated to 190°). For recrystallisation 0·3 gram of the salt was dissolved in 4—5 c.c. of 90 per cent. alcohol, by warming on the water-bath, and, after cooling, ether was added in quantities of a few drops at a time.

The salt is very unstable and therefore difficult to purify.

Determination of chlorine:

1. By precipitating the solution in 90 per cent. alcohol with alcoholic silver nitrate:

0·1055 gave 0·0229 AgCl; $Cl = 5·35$.

2. By Carius' method:

0·1478 gave 0·0348 AgCl; $Cl = 5·8$.

$C_{35}H_{41}O_6N_5,HCl$, requires $Cl = 5·35$ per cent.

Normal Ergotoxine Oxalate $(C_{35}H_{41}O_6N_5)_2,H_2C_2O_4$.

By adding a solution of oxalic acid in alcohol to a solution of ergotoxine in ether, so that the base is in excess, the normal oxalate was formed. The precipitate was washed and dried, and crystallised by gradually adding dry ether to a cold concentrated solution of the salt in 80 per cent. alcohol. After each addition of ether the solution was set aside for crystallisation. If absolute alcohol is used, crystals are much more difficult to obtain.

The salt forms elongated, rectangular plates, showing straight extinction and melting at 179° (the bath being previously heated to 170°). It is soluble in five parts of boiling absolute alcohol, and in twelve parts of alcohol at 25°.

0·1579 gave 0·3727 CO_2 and 0·0949 H_2O. C = 64·35 ; H = 6·72.
0·1168 ,, 0·2735 ,, ,, 0·0652 ,, C = 63·83 ; H = 6·24.
0·1920 ,, 14·4 c.c. N at 15·5° and 768 mm. N = 10·70.
0·2040 ,, 18·3 c.c. N at 13° and 771 mm. N = 10·76.
$(C_{35}H_{41}O_6N_5)_2,H_2C_2O_4$ requires C = 64·24 ; H = 6·29 ; N = 10·42. per cent.

The oxalic acid was determined by adding ammonia, extracting with ether, and precipitating the oxalic acid in the aqueous solution with calcium acetate.

0·203 gave 0·0077 CaO. $H_2C_2O_4$ = 5·7.
$(C_{35}H_{41}O_6N_5)_2,H_2C_2O_4$ requires $H_2C_2O_4$ = 6·6 per cent.

Acid Ergotoxine Oxalate, $C_{35}H_{41}O_6N_5,H_2C_2O_4$.

This salt is formed by shaking a solution of the alkaloid in xylene with excess of a 1 per cent. solution of oxalic acid. On crystallisation from alcohol and acetone it gave minute prisms, melting at 179° with decomposition. It does not crystallise so well as the normal oxalate.

0·2280 gave 0·5137 CO_2 and 0·1313 H_2O. C = 61·45 ; H = 6·44.
0·1770 ,, 0·3988 ,, ,, 0·1030 ,, C = 61·44 ; H = 6·51.
0·2322 ,, 0·5256 ,, ,, 0·1324 ,, C = 61·73 ; H = 6·38.
0·2108 ,, 0·0136 CaO ; $H_2C_2O_4$ = 10·5.
$C_{35}H_{41}O_6N_5,H_2C_2O_4$ requires C = 61·88 ; H = 6·04 ; $H_2C_2O_4$ = 12·6 per cent.

The Transformation of the Ergot Alkaloids into one another.

The transformation of the amorphous alkaloid into the crystalline was first observed by Kraft by boiling a solution of the former in methyl alcohol under a reflux condenser. On cooling, ergotinine

crystallised out. Kraft also observed the formation of hydroergotinine (as he called the amorphous alkaloid), when a solution of ergotinine in 3 per cent. acetic acid is left standing for some days at the laboratory temperature.

Shortly before the publication of Kraft's results, we had independently observed the formation of ergotinine from ergotoxine in an attempt to acetylate the latter alkaloid. With this end in view, 0·64 gram of ergotoxine was boiled with 10 c.c. of acetic anhydride for a few seconds. The solution was then poured into water and stirred vigorously. The solution of the alkaloidal acetates was separated from a small quantity of a dark resin, and the alkaloids were precipitated in the filtrate with ammonia, collected on a filter, and dried in a vacuum. The alkaloidal precipitate weighed 0·49 gram, and required for complete solution about 25 c.c. of boiling absolute alcohol, whereas the ergotoxine employed was soluble in very much less alcohol. On cooling, 0·13 gram of ergotinine separated out, and on concentration of the mother liquor 0·06 gram more, in all therefore about 30 per cent. of the theoretical yield. The identity with natural ergotinine results from the following:

(1) The melting point was 220°, and remained unchanged when the substance was mixed with natural ergotinine.

(2) the composition: 0·1153 gave 0·2895 CO_2 and 0·0668 H_2O. C = 68·48; H = 6·48.

$C_{35}H_{39}O_5N_5$ requires C = 68·91; H = 6·45.

(3) The specific rotation in alcoholic solution:

$$\alpha_D + 0{\cdot}69°\ ;\ l = 1 \text{ dcm.}\ ;\ c = 0{\cdot}200\ ;\ [\alpha]_D + 345°.$$

In a similar experiment the yield of ergotinine was 25 per cent.; for the specific rotation we found:

$$\alpha_D + 0{\cdot}95°\ ;\ l = 1 \text{ dcm.}\ ;\ c = 0{\cdot}245\ ;\ [\alpha]_D + 388°.$$

After boiling the alcoholic solution for half an hour:

$$\alpha_D + 0{\cdot}77°\ ;\ l = 1 \text{ dcm.}\ ;\ c = 0{\cdot}231\ ;\ [\alpha]_D + 326°.$$

The mean value previously found for natural ergotinine after boiling with alcohol was $[\alpha]_D + 328°$.

At first we were unable to repeat Kraft's experiment with methyl alcohol, perhaps because we used ergotoxine prepared by heating the phosphate on the water-bath with ammonia and filtering. Later, at Dr. Kraft's suggestion, we liberated the base with sodium carbonate, extracted with ether, dried the ethereal solution with sodium sulphate, and evaporated it in a vacuum over paraffin wax. We then obtained from 0·139 gram of ergotoxine, dissolved in 2·4 c.c. methyl alcohol after four hours' boiling on the water-bath, 0·031 gram of ergotinine = 23 per cent.

This specimen gave:

$$\alpha_D + 0{\cdot}90^\circ\ ;\ l = 1 \text{ dcm.}\ ;\ c = 0{\cdot}1244\ ;\ [\alpha]_D + 328^\circ.$$

In a similar experiment, when ammonia was used to liberate the base, we obtained a somewhat smaller yield, and in no case was the transformation even approximately quantitative. As we have already observed, we infer from our experiments on the optical rotation of ergotinine that the alkaloid undergoes decomposition by prolonged boiling in alcoholic solution. Since we originally regarded ergotinine as acetylergotoxine, we attempted to hydrolyse it by means of an acid, and selected phosphoric acid because the phosphate is the most characteristic of ergotoxine salts. 0·3 gram of ergotinine, which would require nearly 20 c.c. of boiling absolute alcohol for solution, was heated on the water-bath with 6 c.c. of alcohol containing $1\frac{1}{4}$ molecular proportions of phosphoric acid. At 70° the ergotinine did not readily dissolve, but after boiling for fifteen minutes solution was complete. On standing overnight, 0·2 gram of minute diamond-shaped plates separated out, consisting of long needles of which each end is symmetrically replaced by two dome faces; the extinction is parallel to the long edge of the crystals. They were found to be the phosphate of an amorphous base. On recrystallisation from alcohol and ether much larger plates were obtained, mostly hexagonal or triangular in shape, and differing completely from the slender prisms of ergotoxine phosphate already described. Nevertheless, both phosphates melted at 186—187°, and a mixture of the two showed the same melting point. This new phosphate was converted into the oxalate, which melted at the same temperature as normal ergotoxine oxalate, and resembled it in crystalline form. Finally, the oxalate was reconverted into the phosphate, which was found to have preserved its original crystalline form. Its identity with ergotoxine phosphate we have not as yet definitely established for want of material, but possibly the one is a racemic modification of the other. Both salts show great physiological activity.

In order to prove the absence of acetyl groups in ergotinine, its solution in 20 per cent. aqueous phosphoric acid was distilled with steam. No acid passed over. On neutralisation and concentration the solution yielded the typical prisms of ergotoxine phosphate.

In conclusion, we wish to acknowledge our indebtedness to Mr. W. C. Reynolds, B.Sc., for much help in the isolation of the alkaloids, and to Mr. A. J. Ewins, B.Sc., for valuable assistance throughout the work.

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