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THE ALKALOIDS OF SOME INDIAN ACONITES (*A. Balfourii*, *A. deinorrhizum* and "Chumbi Aconite")

BY

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CXLI.—*The Alkaloids of Some Indian Aconites* (A. Balfourii, A. deinorrhizum, *and "Chumbi Aconite"*).

By Thomas Anderson Henry and Thomas Marvel Sharp.

In 1922, the Director of the Wellcome Bureau of Scientific Research received from Colonel Kennedy, I.M.S. (retd.), a series of small specimens of drugs collected near and used in the Yerpa Monastery in Lhasa. The only specimen of more than anthropological interest was an aconite root, which was identified by Dr. Stapf of the Royal Botanic Gardens, Kew, as *Aconitum rotundifolium*, Kar. and Kir. As this rare species of aconite has not been examined chemically, Colonel Kennedy was kind enough to obtain a further supply of leaves (360 g.) and roots (1,250 g.), collected on the pass between Sikkim and the Chumbi Valley in Tibet at an altitude of about 12,000 feet. This material we now have good reason to believe is not *A. rotundifolium*, and we propose to call it for the present "Chumbi aconite." Colonel Kennedy suggested that Major F. M. Bailey, C.I.E., Political Resident in Sikkim, might be able to obtain more. Major Bailey sent out collectors on two occasions, but the material obtained, on examination by Dr. Stapf, proved to be of the *A. spicatum* type, and as the authors subsequently found that it contained pseudaconitine, instead of the bikhaconitine characteristic of *A. spicatum*, Dr. Stapf agrees that it may be *A. Balfourii*, Stapf.

The latter on examination at the Imperial Institute (*Bull. Imp. Inst.*, 1906, **4**, 38) was found to contain pseudaconitine, but no detailed evidence for this has been published. The authors are greatly obliged to the authorities of the Imperial Institute for providing them with specimens of the roots then examined and of the alkaloid obtained. The results of this comparison leave no doubt that the alkaloid of *A. Balfourii* is pseudaconitine.

The opportunity has been taken to characterise pseudaconitine more completely and to make a preliminary examination of its oxidation products. For these purposes a considerable quantity of the alkaloid has been prepared from the usual source, *A. deinorrhizum*, Stapf (*A. ferox* var. *atrox*, Wall.). Among the new salts prepared are the *hydrochloride*, which had not hitherto been obtained crystalline, and the *picrate*, which, unlike the picrates of most of the aconitines, crystallises easily. The formula generally accepted for pseudaconitine is $C_{36}H_{49}O_{12}N$. Neither the authors' combustion results nor those on which Wright and Luff (J., 1878, **33**, 151), Dunstan and Carr (P., 1895, **11**, 154; J., 1897, **71**, 350), Freund and Niederhofheim (*Ber.*, 1896, **29**, 852), and Schmidt (*Arch. Pharm.*, 1909, **247**, 240) based this formula are in particularly good agreement with the figures calculated from it. Dunstan and Andrews (J., 1905, **87**, 1636) altered the formula to $C_{36}H_{51}O_{12}N$, which the authors propose to adopt, at least provisionally.

It is common ground with previous investigators that pseudaconitine on hydrolysis furnishes pseudaconine, $C_{25}H_{41}O_8N$, and one molecular proportion each of acetic and veratric acids, and that it contains six methoxyl groups (Freund and Niederhofheim, *loc. cit.*). In the present paper, it is shown that the two remaining oxygen atoms function as hydroxyl groups, since pseudaconine yields a well-crystallised tetra-acetyl derivative, and that the nitrogen is present as a methylimino-group.

Carr has stated that on oxidation with permanganate pseudaconitine yields acetaldehyde and a crystalline substance, m. p. 235° (J., 1912, **101**, 2243). We are able to confirm the formation of acetaldehyde, but the solid oxidation product is a complex mixture, which so far we have not been able to separate satisfactorily into its components.

On oxidation with chromic acid, pseudaconitine yields a crystalline *substance*, $C_{34}H_{45}O_{11}N$, which contains one methoxyl group less than its progenitor, but retains intact the methylimino-group. It is neutral in reaction, but can be induced to form salts which are hydrolysed by water. The weakly basic character of this substance as compared with pseudaconitine may be due to the formation of a carbonyl group next to the nitrogen, by the oxidation of a secondary

alcohol group, in a manner similar to that taking place in the oxidation by nitric acid (see below). On hydrolysis by alkalis, the substance yields acetic and veratric acids and a crystalline product, which should be represented by the formula $C_{23}H_{35}O_7N$, but which so far we have not been able to confirm by analysis.

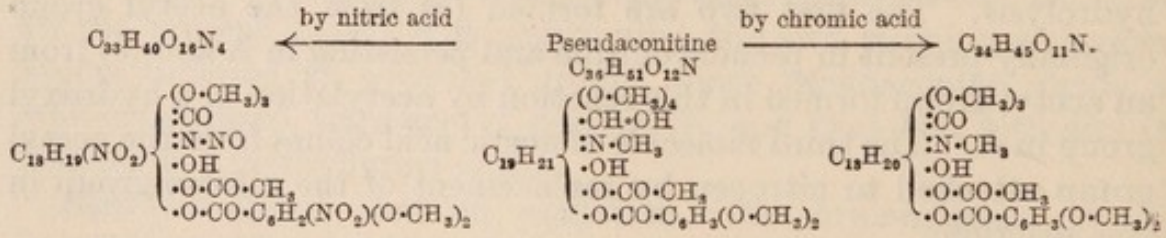
On oxidation with cold nitric acid pseudaconitine yields two products, a pale yellow, crystalline *substance A*, having the formula $C_{33}H_{40}O_{16}N_4$, and a bright yellow *substance B*, $C_{34}H_{43}O_{17}N_3$. Product A still contains the acetyl group, but the veratroyl residue has been nitrated in position 6; the number of methoxyl groups has been reduced to five, two of which belong to the nitroveratroyl radical, so that one has disappeared from the nucleus; the methylimino-group has been converted into a :N·NO group, since the compound gives Liebermann's nitrosoamine reaction; and the remaining nitrogen is probably present as a nitro-group, indicating the presence of a benzene ring in the nucleus. Of the two remaining oxygen atoms, one is present as a hydroxyl group. Evidence for this and simultaneous confirmation of the presence of a :N·NO group was obtained in the following way. The oxidation product A was treated with acetyl chloride at 100° under pressure. The excess of reagent was distilled off, carrying with it nitrosyl chloride formed by the conversion of the :N·NO group into a *N*-acetyl group. The acetylated material contained two substances, of which one was obtained crystalline. This no longer gave the nitrosoamine reaction and on hydrolysis furnished three molecular proportions of acetic acid, *two* by alkaline hydrolysis and *one* by subsequent acid hydrolysis. The first two are formed (*a*) from the acetyl group originally present in pseudaconitine and persisting in A and (*b*) from an acetyl group formed in this reaction by acetylation of a hydroxyl group in A. The third molecule of acetic acid comes from the acetyl group attached to nitrogen by replacement of the nitroso-group in this reaction.

This still leaves one oxygen atom in A to be accounted for. Its function is shown by the behaviour of the crystalline *substance*, $C_{22}H_{31}O_{10}N_3$, formed along with acetic and 6-nitroveratric acids by the alkaline hydrolysis of A. On solution in methyl alcohol containing sodium methoxide, it is converted into a crystalline *sodium* salt, $C_{22}H_{32}O_{11}N_3Na$, produced by addition of the elements of a molecule of sodium hydroxide, which clearly implies the presence of a lactim group in this hydrolytic product and also in A. This indicates that in pseudaconitine there is present a group such as ·NMe·CH·OH, which is converted by treatment with nitric acid into the structure ·N(NO)·CO·, and this on treatment with alkali passes into ·NH(NO):CO·ONa. That a considerable change in the

state of combination of the nitrogen takes place in this last reaction is clear from the fact that, whereas pseudaconitine, A, and the hydrolytic product of the latter all fail on distillation to yield vapours giving the pyrrole pine-shaving test, this test is readily given by the vapours from the sodium salt, and from the ill-defined materials formed by the action of hydrochloric acid, thionyl chloride, and various oxidising and reducing agents on the hydrolytic product. The production of volatile pyrrole derivatives in this fashion does not of necessity imply the presence of a pyrrole nucleus in pseudaconitine or its proximate degradation products now described, but merely that in the hydrolytic product of A the nucleus is in such a form as to yield pyrrole derivatives readily in the somewhat drastic process of dry distillation.

The examination of the bright yellow nitric acid oxidation product B has not been carried very far. On hydrolysis it furnishes acetic and 6-nitroveratric acids and an amorphous nitrogenous product : it contains five methoxyl groups, of which two are in the nitroveratroyl residue, and a nitroso-group which is eliminated on treatment with acetyl chloride as in the case of product A, but neither in this nor in any of the numerous reactions tried on this substance has any well-defined crystalline product been obtained.

In the formation of the chromic acid oxidation product, $C_{34}H_{45}O_{11}N$, and of the nitric acid oxidation product A, $C_{33}H_{40}O_{16}N_4$, very little effect has been produced on the nuclear structure of pseudaconitine, as will be seen from the following tabular summary of what is known regarding the three substances :



The products obtained, moreover, are not promising for gradual further degradation owing to their tendency to produce soluble colloidal substances, and other methods of dealing with the problem are now being investigated.

Most of the substances described in this paper have proved difficult to analyse by combustion, but the hydrolytic products of the chromic acid oxidation product and of the substance A were particularly troublesome in this respect, and we have been fortunate in having the analyses on which we have based the formula, $C_{22}H_{31}O_{10}N_3$, confirmed by Professor R. Robinson, who had microanalyses made in his laboratory by Mr. A. Bennett, for which we take this opportunity of expressing our thanks.

Considerable difficulty has also been experienced in getting satisfactory determinations of nitrogen in pseudaconitine and its degradation products, due, no doubt, to the ease with which these substances evolve methane (Dunstan and Carr, P., 1896, **12**, 48). This difficulty has probably been encountered by other workers, as no determination of nitrogen in pseudaconitine appears to have been recorded since that due to Wright and Luff (*loc. cit.*). This difficulty has been overcome by using a coil of oxidised copper beyond the reduced copper spiral of the ordinary Dumas method, as recommended by Pregl in his micro-analytical method.

Examination of the alkaloid from the " Chumbi aconite " collected by Colonel Kennedy indicates that it is one of the possible acetylbenzoylaconines, of which the two best known are aconitine from *A. Napellus* and japaconitine, obtainable from various species of aconite found in Japan. Most of the recent work on these two alkaloids indicates that they are not identical, but isomeric. The difference between them may depend on the relative positions of the two acyl groups, since they both appear to yield the same aconine, but as they also both furnish, on oxidation by permanganate, the same oxonitine, in which the two acyl groups are still present, they may be merely stereoisomeric. Aconine must contain five hydroxyl groups, since aconitine yields a triacetyl derivative (Dunstan and Carr, J., 1895, **67**, 462), and this, by the replacement of two hydroxyls at a time by two acyloxy-radicals, admits of the existence of a large number of isomerides, which may differ but little in properties. In two recent papers, Majima and his colleagues (*Ber.*, 1924, **57**, 1466) have shown that aconitine (Merck) consists of two isomerides A and C and from seven species of *Aconitum* found in Japan six isomerides have been isolated (*ibid.*, p. 1456), none of the species yielding less than two and most of them four. Japaconitine (Merck), according to these authors, is a mixture of four of these isomerides. The evidence so far brought forward is scarcely sufficient to justify the view that these eight acetylbenzoylaconines are all different, but theoretically there is no reason to doubt their existence. The isomeride now found in the " Chumbi aconite " on the whole resembles aconitine rather than japaconitine (Dunstan and Read, J., 1900, **77**, 45), but this may be due to the fact that, although much work has been done on aconitine, it is not yet a well-characterised alkaloid. Until a further supply of the roots can be obtained the exact relationship of this alkaloid to the other acetylbenzoylaconines cannot be finally established. It is of interest to note that in Stapf's classification of Indian aconites *A. rotundifolium* is placed with the non-poisonous " anthora " group. The " Chumbi aconite " collected by Colonel Kennedy cannot therefore be derived from

A. rotundifolium and cannot be identical with the material originally obtained by Colonel Kennedy at the Yerpa Monastery. Dr. Stapf is of the opinion that the " Chumbi aconite " is allied to *A. spicatum*, but it cannot be definitely identified until more complete botanical material is available. It is of special interest since it is the first recorded instance of an acetylbenzoylaconine being found in an Indian aconite, for although various Indian species have at different times been confused with *A. Napellus*, it seems to be well established now that the latter species has not been found in India.

Dr. Stapf places *A. Balfourii* with *A. deinorrhizum* and, as is now shown, both these species contain the same alkaloid, pseudaconitine.

We should like to take this opportunity of thanking Colonel Kennedy for his kindness in placing his material at our disposal, Dr. Stapf for his unfailing help in identifying botanical material, and Colonel Gage, late Director of the Botanical Survey of India, Major Bailey, Political Resident in Sikkim, and Mr. W. J. Lambert, Conservator of Forests, Jammu and Kashmir State, for their kind assistance in the collection of material.

Experimental.

Aconitum Balfourii *and* A. deinorrhizum.

Extraction of Alkaloids.—The finely ground, air-dry roots were percolated to exhaustion with cold 95% alcohol, the percolate concentrated to low bulk under reduced pressure, and the alkaloids extracted from the residue with 0·5% hydrochloric acid. The acid solution (*A*), after agitation with ether to remove traces of acids, resin, and oil, was made alkaline with sodium carbonate solution and extracted with ether, so long as this continued to remove alkaloid, and after that with chloroform, which took out all but traces. On the removal of the solvent from the ethereal solution pseudaconitine crystallised out. The *A. Balfourii* roots yielded 1·2% of total alkaloid, of which 0·4%, expressed on the air-dry roots, was crystalline. This procedure was modified in working up larger quantities of *A. deinorrhizum* roots. Here the dilute hydrochloric acid extract (*A*) was filtered and the alkaloidal hydrochlorides were extracted by long-continued agitation with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, and an aqueous solution of the residue left after removal of the chloroform was treated with charcoal and filtered into a 50% excess of sodium carbonate solution with constant stirring. The precipitated alkaloids (yield, 0·9% expressed on the air-dry roots) were then collected, washed with water, and dried in a vacuum. From this dry precipitate, crude pseudaconitine was obtained by boiling it with twice its weight of 95% alcohol and filtering the hot solution;

the alkaloid then crystallised on cooling. The yield was 0·4% expressed on the air-dry roots. The acid liquor (*A*) and the sodium carbonate precipitation liquors still contained some alkaloid, which was recovered in the usual way and added to the residues left from the crystallisation of the crude pseudaconitine. The nature of the alkaloids in these residues is being investigated.

Pseudaconitine and Salts.—In the following description the melting points are corrected and, unless otherwise stated, are also decomposition points. The melting points and specific rotations recorded for salts refer to the salt dried to constant weight. The crude alkaloid was recrystallised from hot 95% alcohol until of constant melting point, 212—213°. It was further purified through the hydrobromide, and the base regenerated from the pure salt had m. p. 214°, $[\alpha]_D^{20°}$ + 17·06° (in alcohol; $c = 1·18$) and + 22·75° (in chloroform; $c = 4·76$). The recorded m. p.'s for pseudaconitine vary from 201° (D. and C.) to 212° (F. and N.): for $[\alpha]_D$ Dunstan and Carr found + 18·6° ($c = 1·12$, in alcohol) (Found: C, 62·4, 62·7, 62·8; H, 7·8, 7·9, 7·95; N, 2·2; MeO, 27·2; NMe, 2·7. $C_{36}H_{51}O_{12}N$ requires C, 62·7; H, 7·5; N, 2·0; 6MeO, 27·0; NMe, 4·2%).

The following salts are already known and were prepared for identification of the alkaloid from *A. Balfourii*.

The hydrobromide separates from alcohol in rosettes of triangular prisms, m. p. 199°, $[\alpha]_D^{20°}$ − 18·5° (in water; $c = 2·56$) (Found for air-dry salt: loss at 100° in a vacuum, 6·6. $C_{36}H_{51}O_{12}N,HBr,3H_2O$ requires H_2O, 6·6%. Found in dry salt: Br, 10·25. Calc.: Br, 10·4%). The nitrate crystallises from dry alcohol in triangular prisms, m. p. 198°, $[\alpha]_D^{18°}$ − 17·95° (in water; $c = 3·9$) (Found for air-dry salt: loss at 100° in a vacuum, 1·9. Calc. for $C_{36}H_{51}O_{12}N,HNO_3,H_2O$: H_2O, 2·3%. Found for dry salt: C, 56·9; H, 6·7; MeO, 24·75; NMe, 3·4. Calc. for $C_{36}H_{51}O_{12}N,HNO_3$: C, 57·4; H, 7·0; 6MeO, 24·75; NMe, 3·9%).

The hydriodide separates from hot dilute alcohol in truncated prisms, m. p. 230° (Found for air-dry salt: loss at 100° in a vacuum, 2·5. Calc. for $C_{36}H_{51}O_{12}N,HI,H_2O$: H_2O, 2·2%).

The chloroaurate had m. p. 233°.

The data recorded above for these four salts agree as well as can be expected with those recorded by previous investigators: it is well known that the melting points of the "aconitines" and their salts vary with the rate of heating and the small discrepancies in both directions now found are no doubt due to this cause. Differences in the amounts of water of crystallisation found in the hydrobromide (3 instead of $2H_2O$) and nitrate (1 instead of $3H_2O$) from those recorded by Dunstan and Carr (*loc. cit.*) are due to the

fact that these salts are efflorescent and the amount of water found depends on the conditions under which they are rendered "air-dry."

The following salts are new. The *hydrochloride* was prepared by neutralising the pure base, dissolved in dry alcohol, with hydrochloric acid. The solution was then evaporated to dryness under reduced pressure, the residue dissolved in dry alcohol, and ether added; the salt then separated in twinned triangular prisms and was recrystallised from dry alcohol. It melts at 179—182° and has $[\alpha]_D^{20°} - 18{\cdot}1°$ (in water; $c = 3{\cdot}37$). When the crystals are allowed to dry in air for a short time, they appear to contain 4 molecules of water (Found: loss in a vacuum at 120°, 10·3. $C_{36}H_{51}O_{12}N,HCl,4H_2O$ requires H_2O, 9·0%), but on longer exposure to air efflorescence occurs and the product is represented by the formula $C_{36}H_{51}O_{12}N,HCl,3H_2O$ (Found: loss at 120° in a vacuum, 7·2. Calc.: 6·9%. Found for dry salt: C, 59·05; H, 7·2; Cl, 5·1. Calc. for $C_{36}H_{51}O_{12}N,HCl$: C, 59·5; H, 7·2; Cl, 4·9%). The hydrochloride is very soluble in water and in wet alcohol and this no doubt accounts for the failure of previous workers to obtain it.

The *oxalate*, similarly prepared, crystallises from hot, dry alcohol in minute, transparent, facetted cubes, m. p. 216°. The *perchlorate*, obtained as a crystalline precipitate on addition of sodium perchlorate to a solution of pseudaconitine hydrochloride in water, crystallises from dilute alcohol in needles, m. p. 239° (Found: C, 54·5; H, 7·2. $C_{36}H_{51}O_{12}N,HClO_4$ requires C, 54·7; H, 6·6%). The *picrate* crystallises from alcohol in spheroidal masses of thin, orange-yellow prisms, m. p. 196°.

Pseudaconine.—A quantity of pseudaconine was prepared by hydrolysis of the residues left from the initial crystallisation of pseudaconitine (p. 1110).

It is obtained from acetone in well-defined, large, colourless prisms containing 1 mol. of solvent, m. p. 93—94°, $[\alpha]_D^{20°} + 38{\cdot}7°$ (in water; $c = 1{\cdot}69$) (Found in air-dried substance: C, 62·1; H, 8·8; loss at 120° in a vacuum, 10·4%. Calc. for $C_{25}H_{41}O_8N,C_3H_6O$: C, 62·1; H, 8·75; C_3H_6O, 10·7%). Dunstan and Andrews (J., 1905, **87**, 1629) give m. p. 86—87° (after crystallisation from acetone), $[\alpha]_D = + 39{\cdot}0°$ ($c = 1{\cdot}8$ in water). A *tetra-acetyl* derivative was obtained by heating solvent-free pseudaconine (1 g.) under reflux with acetyl chloride (2 c.c.) for an hour. The acetyl chloride was removed by distillation and the residue dissolved in water and made alkaline with sodium carbonate; the white precipitate thus formed crystallised from ligroin in colourless, stout needles, m. p. 228°, $[\alpha]_D^{20°} - 8{\cdot}1°$ (in alcohol; $c = 0{\cdot}956$) (Found in substance dried at 120° in a vacuum: C, 60·5; H, 7·6; MeO, 19·35; NMe, 4·1. $C_{33}H_{49}O_{12}N$ requires C, 60·8; H, 7·6; 4MeO, 19·05; NMe, 4·5%). The acetyl

groups were estimated by hydrolysis with alcoholic potassium hydroxide, followed by acidification, steam distillation, and titration of the distillate (Found : acetic acid, 37·7. Calc. for four acetyl groups, 36·85%). Pseudaconine was recovered from the steam distillation mother-liquor by extraction with ether after addition of sodium carbonate in excess, the yield of pure crystalline base being 88% of that theoretically possible. The acetyl derivative, although alkaline to litmus, does not form salts and may be crystallised unchanged from solutions in alcoholic sulphuric or oxalic acid. Tetra-acetylpseudaconine causes no tingling sensation when applied to the lips. According to Dr. Trevan, Pharmacologist to the Wellcome Physiological Research Laboratories, it is not toxic to mice on subcutaneous injection in doses of 5 mg.

Oxidation of Pseudaconitine.

(1) *With Nitric Acid.*—Pseudaconitine (5 g.), treated with 30 c.c. of nitric acid (*d* 1·42) at room temperature, slowly dissolved with evolution of brown fumes and rise of temperature to about 45°. After 6 hours the clear solution was poured into water (300 c.c.); the white precipitate, which soon turned yellow, was filtered off, washed with water, and dried over-night in a vacuous desiccator; yield, 4·8 g. The aqueous filtrate, after being nearly neutralised with sodium carbonate, yielded on extraction with chloroform a further 0·5 g. This product was boiled with alcohol, which separated it into a sparingly soluble, *pale yellow substance* (*A*) and a readily soluble, *bright yellow solid* (*B*).

Oxidation product A. This substance is insoluble in water and most organic solvents, and sparingly soluble in hot acetic acid or acetone. From the latter it separates in faintly yellow prisms, which blacken at about 260° and decompose sharply at 270° (Found : C, 52·7, 52·8; H, 5·3, 5·3; N, 7·4, 7·5; MeO, 19·9, 20·6; NMe, 0. $C_{33}H_{40}O_{16}N_4$ requires C, 52·9; H, 5·4; N, 7·5; 5MeO, 20·7%). Acetic acid was estimated by the method described for tetra-acetyl pseudaconine (above) (Found : acetic acid, 8·0, 8·8. Calc. for 1 mol. produced on hydrolysis, 8·0%). A quantitative reduction with titanous chloride was carried out as follows : a solution of a weighed quantity of the substance in concentrated sulphuric acid was set aside for 3 hours, poured into water, and diluted to a definite volume. Aliquot parts of this solution were treated with excess of standard titanous chloride and boiled for 15 minutes in a current of carbon dioxide in a flask fitted with a Bunsen valve. After cooling, the excess of titanous chloride was titrated with standard iron alum solution, potassium thiocyanate being used as indicator. In this way, 16·54 and 16·61 mols. of titanous chloride were used. In view

of the large undetermined part of the molecule, these results are not regarded as conclusive, but they possibly indicate the presence in the molecule of one nitroso- and two nitro-groups (calc., 16 mols. $TiCl_3$). Further, the substance gives Liebermann's nitrosoamine reaction, and evidence is adduced later of the elimination of a nitroso-group when the substance is acetylated. Proof of the presence of one nitro-group is furnished later, in the production of 6-nitroveratric acid on hydrolysis, and the third nitrogen can hardly be in any other condition than that of a nitro-group.

Acetylation. The substance A is unaltered by boiling with acetic anhydride. On heating A (8 g.) with acetyl chloride (32 c.c.) in a sealed tube at 100° for 1 hour, an orange-coloured liquid was obtained. The excess of acetyl chloride was removed by distillation, leaving a white solid, which was twice evaporated to dryness with dry alcohol (yield, 8·45 g.). The acetyl chloride distillate was orange in colour and was fractionally distilled, practically all the colour passing over in the first few c.c. On addition of water to these first runnings the colour disappeared and the liquid gave all the usual reactions for nitrous acid. The colour is therefore evidently due to the presence of nitrosyl chloride formed by the action of acetyl chloride on a nitrosoamine group in A. The white solid is a mixture of at least two substances, one of which can be obtained in well-defined, small prisms by taking advantage of the fact that it is less soluble in boiling *iso*propyl alcohol than the remaining reaction products. For example, the crude material was boiled with *iso*propyl alcohol (100 c.c.), and the solution filtered hot from the undissolved part; the latter was again boiled with the solvent (25 c.c.), the solution was filtered hot, and the residue was dissolved in a large volume of *iso*propyl alcohol, placed on a large boiling water-bath, and left to cool slowly. Crystallisation in this fashion was repeated until the *acetyl* derivative (4 g.) had a constant melting point of 227—230°. The remaining product of the reaction has not been obtained crystalline. The crystalline product does not give Liebermann's nitrosoamine reaction, so the original nitroso-group has been replaced by an acetyl group, since nitrosyl chloride is formed in the reaction and acetic acid is produced on acid hydrolysis (see below) (Found: C, 55·3, 54·9; H, 6·1, 6·15; N, 5·4; MeO, 19·25. $C_{37}H_{45}O_{17}N_3$ requires C, 55·3; H, 5·65; N, 5·2; 5MeO, 19·3%). Hydrolysed by alcoholic potassium hydroxide in the manner described for tetra-acetylpseudaconine (p. 1113), it furnished two molecules of acetic acid (Found: acetic acid, 16·7. Calc. for two *O*-acetyl groups, 14·95%). The residue from the steam distillation gave 6-nitroveratric acid on extraction with ether. The

mother-liquors were neutralised, and evaporated to dryness under reduced pressure, and the residue was boiled with absolute alcohol, which dissolved the third product of the reaction. Numerous attempts to obtain this new substance in a crystalline condition failed, and in order to prove the presence of an *N*-acetyl group, already suggested by the formation of nitrosyl chloride, the crude material was boiled under reflux for 6 hours with 1% hydrochloric acid and steam-distilled, and the silver salt of the volatile acid was prepared in the usual fashion and shown to be silver acetate (Found : Ag, 64·5. Calc., 64·7%). The high figure found for acetic acid in the alkaline hydrolysis is no doubt due to partial hydrolysis of the *N*-acetyl group during the steam distillation of the acid liquor.

Hydrolysis of A. The oxidation product A (3·5 g.) slowly dissolved when mixed with alcohol (70 c.c.) and potassium hydroxide (1·75 g.), dissolved in a little water. After 2 hours the solution was diluted with water (3 vols.), neutralised with sulphuric acid, and filtered from a little potassium sulphate, and the alcohol was removed under reduced pressure on a water-bath. After cooling, the solution was made acid with sulphuric acid and steam-distilled, the steam-distillate was neutralised with sodium hydroxide and evaporated to dryness, and a solution of the residue in a little water was filtered and treated with silver nitrate. The precipitated silver salt was dried over sulphuric acid in a vacuous desiccator (Found : Ag, 64·3. Calc. for silver acetate : Ag, 64·7%). From the liquor left after steam distillation, ether extracted a yellow acid, m. p. 195° after crystallisation from water (Found : C, 47·2; H, 4·4. Calc. for 6-nitroveratric acid, $C_9H_9O_6N$: C, 47·55; H, 4·0%) and also when mixed with 6-nitroveratric acid (m. p. 194°). Further, the methyl ester of the acid from A, alone or mixed with methyl 6-nitroveratrate, melted at 143·5° (Found for the methyl ester : C, 49·3; H, 4·8. Calc. : C, 49·8; H, 4·6%). The mother-liquors from the ether extraction, on repeated agitation with chloroform, yielded 1·25 g. of a colourless solid. If the liquor has become concentrated during the steam distillation, some of this solid is precipitated on shaking with chloroform. This *hydrolytic product of A* crystallises well from alcohol, benzene, or chloroform in colourless, shining needles containing an indefinite amount of solvent. On heating, it froths at 85—115°, the actual temperature depending on the solvent used, but in no case sharply. If crystallised from water and air-dried, it melts with frothing at 165—170°. When quite dry, it has m. p. 215°, and $[\alpha]_D^{20°} + 30·9°$ ($c = 0·59$, in alcohol). For analysis the substance was dried first at 80° and finally at 140° in a vacuum

(Found: C, 53·2, 53·1, 52·74*; H, 6·2, 6·3, 6·11*; N, 8·34*, 8·32*, 8·3; MeO, 18·9; *M*, cryoscopic in nitrobenzene, 487. $C_{22}H_{31}O_{10}N_3$ requires C, 53·1; H, 6·3; N, 8·45; 3MeO, 18·7%; *M*, 497·3).

Action of sodium methoxide. On treating the substance $C_{22}H_{31}O_{10}N_3$ (1 g.) with excess of a 12% solution of sodium methoxide in methyl alcohol (1·3 c.c.) a syrup formed. This practically all dissolved when stirred with methyl alcohol (10 c.c.), and after a few minutes needle-shaped crystals of the *sodium* salt began to separate; these were collected after 12 hours, washed with a little alcohol, and dried in a vacuous desiccator (0·94 g.) (Found: Na, 4·1. $C_{22}H_{32}O_{11}N_3Na$ requires Na, 4·3%). The salt was unaltered in composition after being boiled with acetone, in which it is sparingly soluble. On heating, the substance gives the pyrrole pine-shaving test. It is very soluble in water, forming a faintly alkaline liquid which does not give precipitates with salts of the heavy metals.

Reduction with titanous chloride. The hydrolytic product of A was reduced in the manner described for the oxidation product itself (p. 1113), but the preliminary treatment with sulphuric acid was omitted, as the compound is soluble in water. It required 10·89, 10·90 mols. of titanous chloride for reduction, indicating the probable presence of a nitro- and a nitroso-group (calc., 10 mols.).

Action of acetylating agents. When the hydrolytic product of A is treated with acetyl chloride in a sealed tube at 100° a white solid is formed which has so far not been crystallised. When boiled with acetic anhydride, it furnishes a well-defined *substance* crystallising from alcohol in prismatic needles, m. p. 257° (decomp.), together with a small amount of a second crystalline compound, decomp. 322°. The main product, which retains the nitrosoamino-group, gives analytical figures (except for methoxyl) agreeing with those calculated for a diacetyl derivative. The change is, however, probably more deep-seated, since the original substance is not regenerated when the compound is hydrolysed (Found: C, 53·4, 53·4; H, 6·25, 6·3; N, 7·5; MeO, 18·9. $C_{26}H_{35}O_{12}N_3$ requires C, 53·7; H, 6·1; N, 7·2; 3MeO, 16·0%). We hope to return to the study of this interesting substance when more material is available.

This hydrolytic product of A does not react with semicarbazide nor with methyl iodide under a variety of conditions. Reduction experiments with hydriodic acid, titanous chloride, and sodium and

* We are indebted to Mr. A. Bennett for these results obtained by Pregl's microanalytical method. Great difficulty was experienced in getting satisfactory combustion results for this substance and the other figures recorded are chosen from some 20 combustion figures, about half of which were in good agreement with the formula.

alcohol did not yield any product which could be characterised. Oxidation with permanganate and with chromic acid gave very soluble substances, which could not be purified. On boiling with concentrated hydrochloric acid an amorphous substance was obtained which formed organogels. Attempts to purify this substance by means of a Berkefeld filter did not lead to any conclusive results; it still contains a methoxyl group, but also contains chlorine, and gives the pyrrole pine-shaving reaction. Treatment in a sealed tube with hydrochloric acid at 170° gave rise to methyl chloride, ammonium chloride, and a black, humus-like acidic substance.

The product of the action of phosphorus trichloride on the hydrolytic product gives the pyrrole pine-shaving test; it yields, on solution in dilute sodium hydroxide and extraction with ether, a small amount of an oily substance, which smells strongly of indole, but does not give colour reactions with Nelson's or Ehrlich's reagent. Extraction with chloroform gives a similar small quantity of an oil with an indole-like but rather sweeter smell. No other product was isolated.

Oxidation product B. The orange-coloured substance B (p. 1113) has not been obtained definitely crystalline. It may be purified by extraction in a Soxhlet apparatus with dry ether, in which it is sparingly soluble, and is thus slowly deposited in the flask as a bright yellow, granular powder, which slowly decomposes at 195° but does not melt. After purification in this way it separates from alcoholic solutions in a granular condition with the decomposition point unchanged (Found: C, 53·35, 53·5; H, 5·6, 5·8; N, 5·2, 5·6; MeO, 20·0, 20·2; MeN, 0; *M*, in camphor by Rast's method, 774. $C_{34}H_{43}O_{17}N_3$ requires C, 53·3; H, 5·7; N, 5·5; 5MeO, 20·3%; *M*, 765·4). It contains a nitrosoamino-group which, like that of oxidation product A, is removed on treatment with acetyl chloride; but no definite acetyl derivative could be obtained. On hydrolysis by alcoholic potash, B yields one molecule each of acetic and 6-nitroveratric acids, which were identified as described already (p. 1115). The other product of hydrolysis is an intractable, amorphous, orange-coloured substance.

(2) *Oxidation with Chromic Acid.*—When a solution of pseudaconitine (5 g.) in 10% sulphuric acid (30 c.c.) was treated with potassium dichromate (3·4 g.) in water (145 c.c.) an orange-coloured, granular precipitate was obtained which dissolved after 3 hours' heating on a water-bath. After cooling, the solution was extracted with chloroform, which removed a dark-coloured substance (4·5 g.) containing chromium. This solid was extracted with 0·5% sulphuric acid and the part undissolved (2·47 g.) was crystallised from alcohol. The

oxidation product was thus obtained in colourless, irregular prisms (2·05 g.), decomp. 255°, $[\alpha]_D^{20°}$ + 67·95° (Found: C, 63·4, 63·6, 63·3; H, 7·1, 7·0, 7·2; N, 2·1; MeO, 24·3, 23·6; NMe, 3·4. $C_{34}H_{45}O_{11}N$ requires C, 63·4; H, 7·05; N, 2·2; 5MeO, 24·1; NMe, 4·5%). The substance is insoluble in water, its solution in dilute alcohol is neutral, but it forms salts in presence of excess of acids. The *hydrochloride* was prepared by dissolving the substance in a considerable excess of 10% hydrochloric acid, evaporating the solution to dryness under reduced pressure, and crystallising the dried salt from a mixture of dry alcohol and dry ether: it was thus obtained in stout, glistening needles, which when quite dry frothed at about 180°; $[\alpha]_D^{20°}$ + 39·5° (in dry alcohol; c = 1·006) (Found: C, 60·1; H, 6·9; Cl, 5·2. $C_{34}H_{45}O_{11}N,HCl$ requires C, 60·0; H, 6·8; Cl, 5·2%). The salt is soluble in cold water, but rapidly dissociates on warming. The *picrate*, prepared in the usual way from the hydrochloride, crystallised from dry acetone, on addition of dry ether, in balls of stout, short, yellow needles, m. p. 229—230° (Found: C, 55·15; H, 5·5. $C_{34}H_{45}O_{11}N,C_6H_3O_7N_3$ requires C, 55·0; H, 5·55%). The *chloroaurate* separates from dry alcohol as a granular, yellow powder which sinters at 175°, decomposes at 185°, and is of anomalous composition (Found: Au, 16·2. $4C_{34}H_{45}O_{11}N,3HAuCl_4$ requires Au, 16·5%).

Hydrolysis. The oxidation product (5 g.) was mixed with alcohol (100 c.c.) and 50% potassium hydroxide solution (10 g.) and left to stand for 24 hours; water (100 c.c.) was then added, and the undissolved solid (1·75 g. of unchanged material) filtered off. The filtrate was neutralised with sulphuric acid and filtered from potassium sulphate, and the alcohol removed by distillation. After cooling, the solution was acidified with sulphuric acid and the precipitate formed was collected (0·48 g.). The filtrate was extracted with benzene, the benzene extract added to the precipitate, and the whole dissolved in hot water, from which veratric acid crystallised in colourless needles, m. p. 187°; it was identified by a mixed melting-point determination. The original filtrate was steam-distilled, and acetic acid identified in the distillate by preparation and analysis of the silver salt (Found: Ag, 63·9. Calc.: Ag, 64·7%). The steam-distillation residue was made alkaline with sodium carbonate and extracted with chloroform, yielding a reddish varnish (1·65 g.), which, when dissolved in dry alcohol and treated with dry ether, crystallised as balls of fine, faintly pink needles, m. p. 175—177°. This substance should have the composition $C_{23}H_{35}O_7N$, but it has proved difficult to get consistent results with it on analysis and the material clearly needs further investigation.

"Chumbi Aconite."

The total alkaloid was extracted separately from the roots and leaves by the process described for *A. Balfourii* (p. 1110). The roots yielded 1·1%, and the leaves 0·2%, of total alkaloid, of which one-half and one-quarter, respectively, were obtained as crude crystalline alkaloid melting at 197°. This was converted successively into the hydrobromide, hydriodide, and hydrochloride, each of these salts being recrystallised until of constant melting point. Finally, the pure base (3 g.) was regenerated from the hydrochloride and crystallised from alcohol; it then formed thin, hexagonal plates, m. p. 203° (corr.; decomp.), $[\alpha]_D^{20°}$ + 16·3° (in chloroform; $c = 3·84$). It was carefully compared with (1) Dunstan and Read's japaconitine, of which a small specimen was kindly provided by the authorities of the Imperial Institute, and (2) a specimen of carefully purified aconitine made from *A. Napellus* grown in England. The melting points of the three alkaloids taken simultaneously were 203°, 203°, 204°, respectively. Mixtures of the first two showed no depression, and either of the first two mixed with the third depressed the melting point of the latter, but only to 203°. The melting point of aconitine from *A. Napellus* is generally stated to be 197—198°, but this refers in nearly all cases to Merck's aconitine, which, according to Majima and his colleagues (*loc. cit.*), consists mainly of an alkaloid (aconitine-A), m. p. 202—203°, $[\alpha]_D^{14°}$ + 18·1° in chloroform. The only other difference noted between the alkaloid of "Chumbi aconite" and that of *A. Napellus* was that the former appeared to be more soluble in ether. On analysis the two alkaloids, after drying at 110° in a vacuum, gave the following results:

"Chumbi aconite"	(1)	base: C, 62·6, 62·5; H, 7·6, 7·5; N, 2·2%.
	(2)	hydrobromide: C, 55·6; H, 7·0; Br, 10·5, 10·8%.
A. Napellus	(1)	base: C, 63·1; H, 7·4%.
	(2)	hydrobromide: C, 56·0; H, 7·1; Br, 11·6%.

Calc. for $C_{34}H_{47}O_{11}N$: C, 63·2; H, 7·3. Calc. for $C_{34}H_{47}O_{11}N$,HBr: C, 56·2; H, 6·7; Br, 11·0%.

Calc. for $C_{34}H_{49}O_{11}N$: C, 63·0; H, 7·6. Calc. for $C_{34}H_{49}O_{11}N$,HBr: C, 56·0; H, 6·9; Br, 11·0%.

There is naturally little to choose between these formulæ, but the results for the alkaloid from Chumbi aconite agree better with the figures required by $C_{34}H_{49}O_{11}N$, the formula assigned by Dunstan and Read (*loc. cit.*) to japaconitine, than with $C_{34}H_{47}O_{11}N$, which is the formula accepted for aconitine from *A. Napellus* (compare Majima, *loc. cit.*).

The principal constants of the alkaloid from Chumbi aconite (C)

and its salts are given in the following table and compared with those usually accepted for aconitine (A) and japaconitine (J) and their salts. The melting points and the specific rotations given are for the anhydrous substance in each case, unless stated otherwise, and the solvents are chloroform for the bases and water for the salts. Figures shown in parentheses are new determinations made in the course of this work with the English aconitine already referred to above.

	Bases.			Hydrobromides.		
	C.	A.	J.	C.	A.	J.
M. p. ...	203°	197—198° (204°)	203°	175°	(176—180°)	172—173°
$[\alpha]_D$	+16·3°	+14·6° (+17·3°)	+20·26°	−34·8°	−30·8° (−29·1°)	—

	Hydrochlorides.			Hydriodides.		
	C.	A.	J.	C.	A.	J.
M. p.	174°	149° * (174°)	149° *	225°	226°	195°
$[\alpha]_D$	− 35·9°	−35·4° (−29·2°)	−25·1°	—	—	—

* Probably hydrated salts.

It will be seen that all three alkaloids have similar constants and that the alkaloid from Chumbi aconite more closely resembles aconitine than it does japaconitine. With regard to the discrepancies between the pair it may be pointed out that, although 197—198° is usually accepted as the melting point of aconitine, the specimen of aconitine used in this instance had m. p. 204°, which is close to that (202—203°) recorded by Majima and his co-workers for the aconitine-A they isolated from Merck's aconitine (m. p. 197—198°). The melting point recorded for aconitine hydrobromide varies from 163° to 206—207°. In the authors' experience and under the conditions maintained throughout their determinations, it melts when quite dry at 176—180°; if the dry salt is left in the air for a few minutes, some sintering occurs at 160—163°, and if the temperature is then slowly raised the salt never really melts but decomposes above 200° (compare Schulze, *Arch. Pharm.*, 1906, **244**, 170). The hydrobromide of the alkaloid from Chumbi aconite behaves similarly, but differs in melting quite sharply at 175° under the standard conditions and when quite dry. In recrystallising this hydrobromide from water, a minute quantity of a sparingly soluble salt, m. p. 199° (corr.), was eliminated. It is possible that the long range of melting point usually attributed to aconitine hydrobromide is due to the salt being a mixture, and it is noteworthy that both forms of Majima's aconitine-A hydrobromide are stated to melt sharply.

The English aconitine used by the present authors gave $[\alpha]_D^{20}$

$-29{\cdot}1°$ for the hydrobromide and $[\alpha]_D^{20°}$ $-29{\cdot}2°$ for the hydrochloride. Unfortunately Majima and his co-workers give no figures for the specific rotations of the salts of their aconitines-A and -C. The chloroaurate of the alkaloid of Chumbi aconite, crystallised from alcohol, melts at 136° (air-dry) and at 152° (dried at 100°). The corresponding melting points for aconitine chloroaurate are 136° and 151—152°.

None of the crystallised alkaloid could be spared for hydrolytic experiments, but the residual amorphous alkaloids (7·8 g.) were hydrolysed by keeping them in alcoholic potassium hydroxide for 2 days. The liquors were worked up as described on p. 1118, the bases being extracted with ether (yield, 1·2 g.) and chloroform (yield, 2·4 g.). Both residues were acetylated in the cold with acetyl chloride and yielded the same crystalline product (1 g.) which, when recrystallised from boiling alcohol, had m. p. 241°, $[\alpha]_D^{20°}$ $-32{\cdot}5°$ (in chloroform; $c = 2{\cdot}625$) (Found: acetic acid, 33·1. Calc. for four acetyl groups in $C_{33}H_{49}O_{13}N$: acetic acid, 35·9%). These figures agree with those (m. p. 241—242°, $[\alpha]_D$ $-33{\cdot}7°$) recorded by Majima, Suginomé, and Morio (*Ber.*, 1924, **57**, 1466, 1472) for the tetra-acetyl derivative of aconine-A derived from both jesaconitine (acetylanisoylaconine) and the aconitine-A isolated from Merck's aconitine. The alkaline aqueous liquid was acidified and shaken with ether, which extracted benzoic acid (m. p. 121°). The residual liquor was steam-distilled, the distillate neutralised by sodium hydroxide, and sodium estimated as the sulphate in the dry residue (Found: Na, 32·4. Calc. for $C_2H_3O_2Na$: Na, 28·0%). This high result was not unexpected, as the acetic acid obtained by the hydrolysis of amorphous residual bases of *Aconitum* species is in our experience never pure.

These observations on the amorphous bases of Chumbi aconite on the whole tend to support the view that the crystalline base of this plant is aconitine.

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