XLV.—*The Alkaloids of the Aconites.* Part IV.
The Alkaloids of Japanese Aconite Roots.

By C. R. ALDER WRIGHT and A. P. LUFF (Appendix by C. R. ALDER
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§ 1. *Isolation of Crystallisable Alkaloid.*

DURING the past two or three years several importations of aconite roots have been made from Japan. Whether these consist of one single species or of several mixed together does not seem to be certain, nor is the nature of the predominant species clear. According to the observations of Mr. E. M. Holmes, the roots met with commercially appear to be wholly or almost entirely of one kind, and that different in certain respects from German *A. Napellus* roots. That the roots hitherto imported are actually uniform in quality and different from

both *A. Napellus* and *A. Ferox* is rendered probable by the results detailed below; but how far this may be due to climatic influences, and how far it indicates difference of species, is at present not very clear.

The observations of most manufacturers and pharmacists who have had occasion to examine cursorily or in detail these Japanese roots, point either to their being richer in active ingredients than the *A. Napellus* and *A. Ferox* roots ordinarily met with in commerce, or to their containing a more active alkaloid. Not only is the dust produced in grinding and pulverising much more irritating than that of ordinary aconite roots, but the effects produced on the operator whilst preparing liniments, &c., from the Japanese root, and the irritating action of the liniment on the skin, are, according to Gerrard, far greater than when ordinary aconite roots are employed, so that it is not safe to substitute Japanese roots for the ordinary kinds in the preparation of pharmaceutical tinctures, &c.

A preliminary examination of these roots was made some two years ago by Dr. Paul and Mr. Kingzett (*Pharm. J. Trans.*, Sept. 1, 1877, 172; *Yearbook of Pharmacy*, 1877, 469), who obtained from nearly 4 kilos. of roots nearly 7·2 grams of alkaloids soluble in ether, partly crystallised, partly varnish-like. This yield, = about 0·18 per cent., is considerably superior to that obtained by one of us from a large batch (2 cwts.) of *A. Napellus* roots, viz., about 0·07 per cent. of alkaloids soluble in ether (Part I, this Journal, 1877 [1], 143); whence it would seem probable that the greater potency of the Japanese roots is due rather to its containing more of the active ingredient than to this latter being intrinsically more energetic physiologically. The small quantity of crystallised substance obtained in a pure condition by Paul and Kingzett prevented their studying the properties of the alkaloid and its derivatives as thoroughly as might be desired. They describe it as a well crystallised base, infusible at 100° (its melting point was not determined), giving the following numbers on analysis: carbon, 62·926; hydrogen, 7·900 and 7·726; nitrogen (by volume) 2·567; and not forming any crystallised salts, although many and varied attempts to prepare them were made. The molecular weight was not controlled by the formation of a gold salt, &c. (probably through insufficiency of material); hence, regard being had to the probable imperfect accuracy of nitrogen determinations by volume, it is evident that the formula deduced by them from the above numbers (viz., $C_{29}H_{13}NO_6$, requiring carbon, 63·38; hydrogen, 7·83; nitrogen, 2·55), can only be regarded as an approximation to the truth, especially as this formula requires not only more hydrogen than that found in one of the two determinations, but slightly more than that given by the mean of these two. As pointed out by one of us during the discussion

on the paper, the numbers obtained and most of the properties of the crystallised alkaloïd are quite compatible with the possibility of this base being simply pseudaconitine, $C_{36}H_{49}NO_{12}$, which requires carbon, 62·88; hydrogen, 7·13; nitrogen, 2·04; numbers almost exactly the same as those deducible from Paul and Kingzett's analysis, after making allowance for the probable excesses of hydrogen and nitrogen found, whilst the difficulty in forming crystallised salts, noticed by Paul and Kingzett, is precisely that noticed with pseudaconitine, the gold salt and nitrate of which are the only salts yet obtained crystallised; the preparation of these crystalline salts, moreover, requires peculiar manipulation, the nature of which was not known at the time of the publication of their results (Part II). On the other hand, the crystallisability of the base obtained by Paul and Kingzett appeared to them to be much higher than that of pseudaconitine, wherefore they were disposed to regard it as a distinct alkaloïd. This view, moreover, is borne out by the fact observed by them, that on boiling their base with dilute sulphuric acid a liquid was obtained capable of reducing Fehling's solution. Now pseudaconine, producible in this way from pseudaconitine, does not reduce Fehling's solution, although aconine, the corresponding saponification-product of aconitine, does so. It hence appears at least probable that the base examined by Paul and Kingzett was a saponifiable one like aconitine and pseudaconitine, in which case a simple explanation is given of the circumstance noticed by Paul and Kingzett, that the mother-liquor of the base when recrystallised from hot dilute alcohol contained an organic salt of an alkaloïd in solution. Doubtless the original alkaloïd became partially saponified by the hot aqueous liquid, just as pseudaconitine does under similar conditions (Part II). Paul and Kingzett, however, considered that the occurrence of a salt in the mother-liquors pointed to the solution by ether of this salt (possibly aconitate) during the extraction of the alkaloïds; a most unlikely thing to happen, considering that the ether was applied in presence of excess of alkaline bicarbonate, which must inevitably have decomposed such a salt, forming the corresponding sodium salt, carbon dioxide, and the free alkaloïd.

Paul and Kingzett having, through various circumstances, relinquished the further investigation of these Japanese aconite roots, and the nature of the contained alkaloïds being thus in an uncertain state, we have, at the request of the Pharmaceutical Conference (to which our thanks are due for several money grants for carrying out the following experiments, as well as those detailed in Parts I, II, and III), made a careful examination of the subject. Our results (obtained with two different batches of roots), as well as some further results (obtained with a third batch by one of us and Mr. A. E. Menke) detailed in an appendix to this paper, indicate that the view held by

Paul and Kingzett that Japanese aconite roots contain an alkaloid different from both aconitine and pseudaconitine, and peculiar to themselves, is correct. Practically identical analytical numbers were obtained, not only with all three batches of roots, but also with each fraction into which each batch of crystallisable alkaloid was fractionated, with a view to finding out whether the substance obtained was a mixture of more than one base. These numbers, whilst not far removed from the percentages obtained by Paul and Kingzett, lead to a formula considerably different from that assigned by them, the molecular weight being much higher. The alkaloid, which thus appears to be the only crystallisable one contained, presents many resemblances to aconitine, and, like that base, splits up into benzoic acid and a derivative closely resembling aconine. This latter saponification-product reduces Fehling's solution, whence it appears probable that the substance extracted by Paul and Kingzett was actually the same as that obtained by us. It is difficult, however, to understand why in that case Paul and Kingzett failed to obtain crystallised salts, as every specimen of base obtained by us readily yielded a well crystallised nitrate hydrochloride and hydrobromide on simply stirring a pulverised crystal in a watch glass with a little dilute acid. Solution to a clear fluid at first took place, and on further stirring, a crystallised magma of the respective salt was formed, just as with aconitine.

The first batch of roots examined by us was obtained from Messrs. Wright, Layman, and Umney: about 14 kilos. of ground root were percolated with alcohol acidulated with tartaric acid, 1 part of acid per 100 of roots being employed: the percolate was evaporated to a small bulk, diluted with water, strained from precipitated resinous matter, shaken with ether whilst still acid to remove remaining resinous matter, and then rendered slightly alkaline with sodium carbonate, and shaken repeatedly with ether, the ethereal solutions being shaken with aqueous tartaric acid, and used over again. After some six treatments with an equal bulk of ether, but little alkaloid was dissolved out, and this little appeared to be non-crystalline; the alkaline liquors, however, contained (as remarked by Paul and Kingzett, and like the corresponding liquors from *A. Napellus*—Part I) some amount of alkaloidal matter in solution; this was examined as described below (§ 5), but appeared to contain only non-crystalline basic matter. The crude tartaric acid solution of bases thus obtained was treated with sodium carbonate and fresh ether, and the ethereal solution allowed to evaporate spontaneously: a copious crop of crystals was obtained with a quantity of viscid alkaloidal matter, readily soluble in alcohol and ether. By filter-pumping and washing the crystals were obtained pretty free from amorphous bases: the filtrates on spontaneous evaporation deposited a few more crystals, too small in quantity for examination; finally there

were obtained about 12 grams of crystallised base and 25 of resinous basic matter from the filtrates, corresponding to about 0.08 per cent. of crystals, and 0.17 per cent. of amorphous bases, = 0.25 per cent. in all, or somewhat more than was obtained by Paul and Kingzett, who obtained a total yield of about 0.18 per cent.

The first crystals deposited were purified by several crystallisations from ether, and were then marked (A): on analysis they yielded numbers close to those required for apo-aconitine, whence we were at first inclined to suppose that the alkaloid originally present was aconitine, and that it had been partially dehydrated during extraction by the tartaric acid: this view, however, was negatived by the fact that just the same numbers were obtained after converting crystals (A) into hydrobromide, recrystallising the salt, and regenerating the alkaloid (Specimen B); whilst no change was introduced by repeating the purification by again converting into hydrobromide, crystallising the salt, and regenerating the base (Specimen C). Moreover the mother-liquors of the first crop of crystals of hydrobromide were treated with sodium carbonate and ether, and the base that crystallised from the ether examined (Specimen D). In all four cases numbers were obtained which, though not far from those required for apo-aconitine, were yet sufficiently removed therefrom to indicate a difference, the carbon and especially the hydrogen found being uniformly higher than the values got on examination of pure apo-aconitine (Part III).

The second batch of roots was worked up by Messrs. Hopkin and Williams in the same way as that above described, being percolated with alcohol acidulated with tartaric acid (1 part per 100 of roots). The bases soluble in ether finally obtained were sent to us without purification as a nearly white amorphous powder; on dissolving this in tartaric acid, adding sodium carbonate, and shaking with ether, the whole dissolved: on spontaneous evaporation about 60 per cent. of the rough alkaloidal mass was obtained as crystals, and 40 as non-crystalline basic matter from the ethereal mother-liquors. The crystals thus obtained after washing with ether were stirred up with warm dilute nitric acid; on standing and adding a little concentrated nitric acid a magma of crystals was formed, which was separated by the pump filter and washed with dilute nitric acid: from these crystals the base was regenerated by soda and ether (Specimen E). The mother-liquors of these crystals were treated with soda and ether, and the crystals that separated examined (Specimen F). Finally, Specimen E was converted into hydrobromide, the salt recrystallised, and the base regenerated by soda and ether (Specimen G).

The numbers obtained with all seven of these specimens are practically identical: each sample was anhydrous when air-dry.

Specimen (A) 0.2770 gram gave 0.6440 CO₂ and 0.1845 H₂O.
 0.3555 „ gold salt gave 0.0730 Au.
 „ (B) 0.2580 „ gave 0.6010 CO₂ and 0.1745 H₂O.
 „ (C) 0.2600 „ „ 0.6045 CO₂ and 0.1735 H₂O.
 0.5670 „ burnt with soda lime gave by titration
 0.01285 N; by platinum salt, 0.0910 Pt.
 0.6780 gram gold salt gave 0.1380 Au.
 „ (D) 0.2755 „ gave 0.6425 CO₂ and 0.1850 H₂O.
 0.4705 „ gold salt gave 0.0965 Au.
 „ (E) 0.2960 „ gave 0.6920 CO₂ and 0.1985 H₂O.
 0.5965 „ gold salt gave 0.1230 Au.
 „ (F) 0.2775 „ „ 0.6500 CO₂ and 0.1850 H₂O.
 0.3360 „ „ 0.7840 CO₂ and 0.2365 H₂O.
 0.1940 „ „ 0.0395 Au.
 „ (F) 0.5340 „ gold salt gave 0.1095 Au.
 „ (G) 0.3035 „ gave 0.7060 CO₂ and 0.2055 H₂O.
 0.5235 „ gold salt gave 0.1060 Au.
 0.3495 „ burnt with soda lime, gave by titration,
 0.00799 N; by platinum salt, 0.056 Pt.

Specimen.	Carbon.	In free base. Hydrogen.	Nitrogen.	In gold salt. Gold.
A	63.41	7.40	—	20.53
B	63.52	7.51	—	—
C	63.41	7.41	{ 2.27 2.27	20.35
D	63.60	7.45	—	20.51
E	63.76	7.45	—	20.62
F	{ 63.88 63.63	7.41	—	20.36
		7.82*	—	20.50
G	63.44	7.52	{ 2.29 2.26	} 20.24
	63.58	7.50	2.27	20.44

The constancy of the numbers obtained with these different fractions apparently excludes the possibility of their being mixtures of two different bases, and hence renders it improbable that they consisted of two bases, C₃₃H₄₅NO₁₁ and C₃₃H₄₃NO₁₀, mixed in about equivalent proportions; for the results obtained with aconitine and pseudaconitine indicate that had this been the case the fractionations would have more or less completely separated these constituents. Such a mixture,

* This combustion was made in the old-fashioned way by mixing with oxide of copper; the others were all done by the breech-loading process, the substance to be burnt being introduced in a platinum boat, and the combustion finished in a stream of oxygen.

however, would very closely correspond with the numbers found, inasmuch as it would require as follows:—

	Calculated for $C_{33}H_{45}NO_{11} + C_{33}H_{43}NO_{10} = C_{66}H_{88}N_2O_{21}$.	Found.
Carbon in base	63·67	63·58
Hydrogen „	7·07	7·50
Nitrogen „	2·25	2·27
Gold in gold salt.....	20·39	20·44

Moreover, had the substances examined been mixtures of two bases, $C_{33}H_{45}NO_{11}$ and $C_{33}H_{43}NO_{10}$, related as aconitine and apo-aconitine or as pseudaconitine and apo-pseudaconitine, prolonged heating with concentrated tartaric acid solution should have converted the mixture wholly into $C_{33}H_{43}NO_{10}$, requiring carbon 64·60, hydrogen 7·01, nitrogen 2·28, Au in gold salt 20·57; but as shown below (§ 2) no change whatever in composition was thus produced, no increase in carbon percentage being caused by the treatment: hence, since no simple mono-nitrogenous formula agrees well with the average numbers found, and since the formula, $C_{66}H_{88}N_2O_{21}$, expresses them perfectly, it results that in all probability this formula expresses the true composition of the single base isolated. It is noticeable that a substance of this composition might be supposed to be formed from a base, $C_{33}H_{47}NO_{12}$, by the removal of water as follows:—

and that this hypothetical parent base, $C_{33}H_{47}NO_{12}$, only differs from aconitine, $C_{33}H_{43}NO_{12}$, by H_4 . The results detailed below (§ 4) as to the action of benzoic anhydride on the crystallised base isolated as above described, and the nature of its saponification-products (§ 3), viz., benzoic acid, and a base containing H_4 more than apo-aconine, go far to indicate that this view of its formation, if not absolutely correct, is not far from the truth: as stated in the Appendix, however, attempts to isolate the hypothetical parent base, $C_{33}H_{47}NO_{12}$, by treating Japanese roots with alcohol not acidulated by any acid at all, so as to avoid as far as possible dehydrating action, did not lead to this result, only the base, $C_{66}H_{88}N_2O_{21}$, being thus obtained.

In order to avoid circumlocution, it is proposed to refer to the crystallised base actually isolated, $C_{66}H_{88}N_2O_{21}$, as *Japaconitine*, and to its saponification-product (§ 3) as *Japaconine*. Should, however, the existence of the hypothetical parent base, $C_{33}H_{47}NO_{12}$, be hereafter substantiated, and the formation of $C_{66}H_{88}N_2O_{21}$ from it by dehydration as above be proved, it perhaps might be more convenient to term the parent base *japaconitine*, and the derivative “sesqui-apo-japaconitine,”

when the saponification-product would become "apo-japaconine" (*vide* § 3 and 4 and Appendix, conclusion).

The seven specimens of japaconitine above described melted in capillary tubes at temperatures lying between 184° and 186° corrected in each case; or from 1° to 2° higher than aconitine tested side by side, and sensibly at the same temperature as apo-aconitine. In Part I, by an uncorrected error of the press, 189° is given as the melting point of aconitine, instead of 183 — 184° .

Japaconitine hydrobromide crystallises, like aconitine hydrobromide, with $2\frac{1}{2}\text{H}_2\text{O}$ per C_{33} present.

1.0925 gram of air-dry hydrobromide from the first batch of roots (the salt from which specimen B was prepared) lost at 100° 0.0685 gram = 6.27 per cent.

0.8575 gram of the salt from which specimen C was prepared lost 0.0560 = 6.53 per cent.

1.0240 gram of the salt from which specimen G was prepared, lost 0.0620 = 6.05 per cent.

Calculated for $\text{C}_{66}\text{H}_{88}\text{N}_2\text{O}_{21}$, 2HBr, $5\text{H}_2\text{O}$ = 6.02 per cent.

§ 2. *Action of Concentrated Tartaric Acid Solution on Japaconitine.*

It has been shown in Parts II and III, that when aconitine and pseudaconitine are heated for some hours with concentrated tartaric acid solution to 100° , the elements of water are removed, and apo-derivatives formed. Japaconitine (Specimen G) was heated to 100° for eight hours with a large excess of nearly saturated tartaric acid solution; on treatment with soda and ether, a base crystallised precisely resembling japaconitine in all respects, and yielding on combustion precisely the same numbers as the original substance; wherefore no dehydration had taken place.

(I.) 0.2750 gram gave 0.6370 CO_2 and 0.1820 H_2O after conversion into crystallised nitrate, filter-pumping, and regenerating by soda and ether.

(II.) 0.2470 gram gave 0.5735 CO_2 and 0.1645 H_2O .

	Calculated for $\text{C}_{66}\text{H}_{88}\text{N}_2\text{O}_{21}$.	Original substance. Specimen G.	I.	II.
Carbon	63.67	63.44	63.17	63.32
Hydrogen	7.07	7.52	7.35	7.40

The melting point was unaltered.

§ 3. *Action of Saponifying Agents on Japaconitine.*

On heating japaconitine with alcoholic potash saponification rapidly takes place, being complete at the end of a few hours; by evaporating

off the alcohol, treating the residue with dilute sulphuric acid and ether, and allowing the ethereal solution to evaporate spontaneously, benzoic acid with a small quantity of resinous feebly acid bye-product is obtained, just as with aconitine (Part III).

0·880 gram from first batch of roots worked up gave of benzoic acid containing a little resinous matter, 0·1730: on titration with decinormal soda this corresponded to 0·1715 $C_7H_6O_2$.

1·9665 gram from second batch of roots gave 0·4410 gram of benzoic acid containing resinous matter; by titration this corresponded to 0·412 $C_7H_6O_2$.

Calculated benzoic acid per 100 parts of japaconitine.	By titration.	Found. By weighing.
19·6	19·5	19·6
—	20·9	22·4

This benzoic acid agreed in all respects with ordinary benzoic acid; it melted at $120\cdot5^\circ$, and gave a silver salt of which

0·2120 gram gave $0\cdot1000\text{ Ag} = 47\cdot17\text{ per cent.}$

Calculated for $C_7H_5AgO_2 = 47\cdot16\text{ per cent.}$

The complementary product was isolated just as aconine from the corresponding substance obtained with aconitine, viz., by alkalisng with sodium carbonate the liquors from which the benzoic acid had been extracted by ether, evaporation to dryness, extraction of alkaloidal matter by alcohol, and purification of the varnish-like mass left on evaporation to dryness by chloroform. It constituted a yellowish-white friable varnish, readily soluble in water, alcohol, and chloroform, all but insoluble in ether, and exhibiting so close a resemblance to aconine in every respect, save analytical numbers, that the two bodies could be distinguished in no other way: it reduces Fehling's solution when boiled therewith.

0·2640 gram gave $0\cdot5745\text{ CO}_2$ and $0\cdot1925\text{ H}_2\text{O}$.

	Calculated.	Found.
$C_{26}\dots\dots\dots$	312	59·20
$H_{41}\dots\dots\dots$	41	7·78
$N\dots\dots\dots$	14	2·66
$O_{10}\dots\dots\dots$	160	30·36
$C_{26}H_{41}NO_{10}$	527	100·00

Converted into mercuriodide, 0·2535 gram gave $0\cdot1600\text{ AgI}$.

	Calculated for $C_{26}H_{41}NO_{10}, HI, HgI_2$.	Found.
Iodine.....	34·35	34·11
		2 E 2

From these numbers it would seem that the saponification of japaconitine is effected in accordance with the equation—

Assuming the existence of the hypothetical parent base, $\text{C}_{33}\text{H}_{47}\text{NO}_{12}$, referred to in § 1, this might be written—

the parent base being then $(\text{C}_{26}\text{H}_{39}\text{NO}_7) \begin{array}{l} \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{OH} \\ \diagup \text{OH} \\ \diagdown \text{OH} \end{array}$.

The experiments described in the next section lend considerable support to this view.

§ 4. *Action of Benzoic Anhydride on Japaconitine and Japaconine.*

It has been shown in Parts II and III that by the action of benzoic anhydride on aconitine, aconine, and pseudaconine, dibenzoylated bodies are formed represented in the case of each of the first two

substances by $(\text{C}_{26}\text{H}_{35}\text{NO}_7) \begin{array}{l} \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{O} \\ \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \end{array}$ (the products being apparently

identical), and by the formula $(\text{C}_{27}\text{H}_{37}\text{NO}_5) \begin{array}{l} \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{O} \\ \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \end{array}$ in the case of

pseudaconine; it, therefore, might be supposed that an analogous substance would be formed in the case of japaconitine; on trying the experiment, however, the product was found to be a substance containing *four* benzoyl-groups for every C_{33} in the original japaconitine; viewing japaconitine, as above, as being derived from a parent base

$(\text{C}_{26}\text{H}_{39}\text{NO}_7) \begin{array}{l} \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{OH} \\ \diagup \text{OH} \\ \diagdown \text{OH} \end{array}$ by dehydration, the tetrabenzoylated body may

be represented as being $(\text{C}_{26}\text{H}_{39}\text{NO}_7) \begin{array}{l} \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagup \text{O} \cdot \text{C}_7\text{H}_5\text{O} \\ \diagdown \text{O} \cdot \text{C}_7\text{H}_5\text{O} \end{array}$, formed by the direct

addition of benzoic anhydride to japaconitine, thus:—

Moreover, the same tetrabenzoylated derivative appears to be produced when benzoic anhydride acts on japaconine, the reaction then being:—

Japaconitine was heated to 100° for eight hours with twice its weight of benzoic anhydride, containing a small amount of fluid impurities, which rendered it liquid at 100°. On adding alcohol to the product and then agitating with a large bulk of ether and some aqueous tartaric acid, a solution of an alkaloidal tartrate was formed, the benzoic acid and impurities present and the excess of anhydride remaining unchanged being retained in solution in the ether. From the tartrate thus obtained, alkalis threw down white flakes, readily soluble in ether, but not crystallising distinctly from that menstruum. Of these,

0·2770 gram dried at 100° gave 0·6825 CO₂ and 0·1685 H₂O.

	Calculated.		Found.
C ₅₄	648	67·43	67·20
H ₅₉	59	6·14	6·75
N.....	14	1·46	—
O ₁₅	240	24·97	—
(C ₂₆ H ₃₉ NO ₇)(O.C ₇ H ₅ O) ₄	961	100·00	

On saponification, 0·4690 gram of this base gave 0·2415 gram of benzoic acid, containing a little resinous matter; on titration this neutralised soda equivalent to 0·235 C₇H₆O₂.

	Calculated.	Found.	
		By weighing.	By titration.
Benzoic acid.....	50·78	51·49	50·11

This benzoylated derivative did not crystallise from alcohol or ether, nor did it form readily crystallisable salts with acids. On stirring

with dilute nitric acid, a very insoluble nitrate was formed, which appeared indistinctly crystalline under the microscope. It exhibited close similarity to benzoyl apo-aconitine; in a capillary tube it began to soften between 130° and 140° , but did not melt thoroughly until a temperature higher than 160° was attained, no distinct melting point being noticeable.

In order to see if the same substance is obtainable on benzoylating japaconine, this base was treated with benzoic anhydride, &c., just as above described; this base finally obtained perfectly resembled that from japaconitine in all respects.

0.2360 gram gave 0.581 CO_2 and 0.145 H_2O .

	Calculated.	Found.
Carbon.....	67.43	67.11
Hydrogen	6.14	6.82

0.4860 gram, saponified with alcoholic potash, gave 0.2540 gram of benzoic acid containing a little resinous matter; on titration, this neutralised alkali equivalent to 0.2470 $\text{C}_7\text{H}_5\text{O}_2$.

Calculated.	Found.	
	By weighing.	By titration.
50.78	52.26	50.82

A marked difference thus exists between japaconitine and aconitine, in that the latter forms on benzoylating only a dibenzoyl derivative; whilst the former gives a tetrabenzoyl derivative (per C_{33} in each case).

§ 5. *Noncrystalline Alkaloids of Japanese Aconite Roots.*

The mother-liquors left on crystallising from ether the crude japaconitine obtained as above described closely resembled the corresponding substance obtained from *A. Napellus* roots (Parts I and III); the varnish thus obtained from the first batch of roots worked up gave the following numbers:—Carbon, 67.69; hydrogen, 7.75; nitrogen (by soda lime), 3.23; whence, as with *A. Napellus*, a base was apparently present of lower molecular weight than japaconitine, and containing a higher percentage of carbon. No crystalline substance could be obtained from either this specimen of varnish or the similar substance obtained from either of the other two batches of roots examined.

The alkaline liquors, from which all alkaloids soluble in ether had been washed out by repeated treatment with that menstruum, still contained a considerable amount of alkaloidal matter, precipitable by mercuriodide of potassium. On treating the precipitate suspended in alcohol with sulphuretted hydrogen, evaporating, dissolving in water, and precipitating with sodium carbonate, a large quantity of flocculent

basic matter was thrown down apparently identical with the amorphous base from the ethereal mother-liquors of japaconitine; the sodium carbonate filtrate, on evaporation to dryness and treatment with alcohol yielded a small quantity of amorphous basic matter, insoluble in water, with some amount of a base readily soluble in water and chloroform, and apparently identical with japaconine. The quantity of amorphous alkaloid obtained from the mercuriodide precipitate corresponded to about 20 grams, or 0.14 per cent. of the weight of the roots employed, and hence was nearly equal to the amorphous alkaloids obtained from the ethereal mother-liquors.

In each case examined, all the crystallisable alkaloid present in the concentrated extract obtained from the roots was dissolved out by four or five successive treatments with ether; the amorphous base or bases present appear to be retained in alkaline fluids much more energetically than japaconitine, so that prolonged treatment with ether fails to dissolve out the whole, although a portion is thus extracted. In this respect the amorphous bases resemble morphine when dissolved in caustic potash, or cotarnine dissolved in sodium carbonate or dilute caustic soda; agitation with ether (and certain other solvents) then only removes a fraction of the dissolved alkaloid.

APPENDIX.

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THE results above described indicate that each of the two different batches of Japanese aconite root examined contained one and only one crystallisable alkaloid, the substance finally obtained being in every case identical, no matter which batch or what fraction was examined. In each instance the roots were originally worked up by percolation with alcohol acidulated with tartaric acid in the proportion of one part of acid to 100 of root; it would seem *à priori* not at all impossible that the tartaric acid thus introduced might act on the alkaloid during the evaporation down of the alcoholic percolate, the result probably being (judging from the action of tartaric acid on aconitine and pseudaconitine) removal of the elements of water without any saponification being effected; in other words, it is not unlikely that the roots originally contained what has been above referred to as the "hypothetical parent base," $C_{33}H_{47}NO_{12}$, and that this became dehydrated forming $C_{66}H_{88}N_2O_{21}$, its sesqui-apo-derivative, during the process of extraction.

In the hope of isolating the unaltered base, $C_{33}H_{47}NO_{12}$, probably originally present, a third batch of roots was worked up, but without any acid at all being employed to acidulate the alcohol used; 1 cwt. of

roots (about 50 kilos.) were percolated by Messrs. Hopkins and Williams with plain alcohol; the percolate, condensed to about $1\frac{3}{4}$ gallons by evaporation, was sent to us and the alkaloids contained isolated as described above, viz., by exposure to air for some days to remove most of remaining alcohol, addition of water, filtration, treatment with sodium carbonate and ether, and agitation of the ethereal extract with tartaric acid solution. From the crude tartrates thus obtained, the crystallised base was isolated by treatment with soda and ether, spontaneous evaporation, and separation of the crystals thus produced from the resinoid base accumulating in the mother-liquors by filter-pumping and washing with ether. After methodical crystallisation, there were ultimately obtained about 50 grams of crystallised base and 55 grams of resinoid base from the mother-liquors, or about 0.10 per cent. of crystals and 0.11 per cent. of varnish-like bases, or 0.21 per cent. in all. As previously indicated by Paul and Kingzett, and as found with the first batch examined, this yield is considerably superior to that obtained with a good sample (2 cwts.) of *A. Napellus* roots (Part I), which gave about 0.03 per cent. of crystallised aconitine and 0.04 per cent. amorphous bases, or 0.07 per cent. in all of alkaloids soluble in ether. From these results, it may be fairly inferred that the Japanese roots are considerably richer in crystallisable as well as total alkaloids than ordinary *A. Napellus* roots; whence, since the action of japaconitine on the skin appears to be much the same as that of aconitine and pseudaconitine, it seems highly probable that the Japanese roots will prove a more convenient source of crystallisable definite active alkaloids than the roots now ordinarily used, the more so as japaconitine is more easily separable from the amorphous bases than is the case with pseudaconitine, which appears at present to be the active constituent of most of the commercial so-called "aconitine."*

The crystals that were thus obtained were got in three different fractions, as follows:—By spontaneous evaporation until about three-quarters of the crystallisable alkaloid present had separated, filter-pumping, and washing with ether, a mass of crystals was obtained almost free from amorphous bases. This was dissolved in tartaric acid, precipitated with a slight excess of clear caustic soda solution, and agitated with ether sufficient to dissolve only about a quarter of the precipitate, which quickly became a mass of crystals. The whole was then filter-pumped and the residue washed with ether, and finally several times with water to remove soda salts; the crystals thus left were marked A. The ethereal solution thus filtered from A gave on spontaneous evaporation a crop of crystals (B), identical in all respects

* Dr. T. R. Fraser, of Edinburgh, has kindly undertaken the comparative examination of the physiological action of aconitine, pseudaconitine, japaconitine, and some of their derivatives.

with A. Finally, the ethereal mother-liquors from which the original mass of crystals had separated, deposited on standing a further quantity of crystals with much viscid alkaloïd; by thinning with ether and filter-pumping, the crystals were mostly separated from the amorphous alkaloïd; the crystals were finally purified by rubbing with dilute warm nitric acid, which at first dissolved them, and then deposited a crystalline nitrate. From this crystallised salt, drained on the pump-filter, and washed with dilute nitric acid, the base was isolated by soda and ether, and the crystals thus obtained were marked C.

A fourth specimen of crystals (D) was obtained as follows:—In order to see whether all the crystallisable alkaloïds can be practically removed from the ground roots by alcohol alone, the marc practically exhausted by plain alcohol was treated by Messrs. Hopkins and Williams with a further quantity of alcohol, slightly acidulated with sulphuric acid (about 30 grains of concentrated acid per gallon); the percolate was evaporated down to about a gallon, and sent to us for examination. On treatment in the same way as the first percolate with plain alcohol, it was found, firstly, that only a small quantity of resinous matter was present; wherefore the plain alcohol first used had evidently dissolved out practically all the resinous matter from the roots. Secondly, the total amount of alkaloïds soluble in ether after repeated treatments only amounted to between 2 and 3 grams, of which about half was crystalline and half amorphous; the crystals left on separation by the filter-pump and washing with ether were recrystallised from ether, and thus formed specimen D, identical in all respects with A, B, and C. It is thus evident that alcohol alone, without addition of any acid, will suffice to remove practically all the alkaloïds contained in Japanese roots (and presumably, therefore, also in *A. Napellus* and *A. Ferox* roots). It is noticeable that the condensed percolate obtained with plain alcohol was distinctly acid to test paper; whence, since inorganic acids are apt to decompose and saponify the aconite alkaloïds, and tartaric acid to dehydrate them, it appears to be preferable to exhaust aconite roots with plain alcohol rather than with acidulated alcohol in manufacturing the pure crystallised alkaloïds, which it is to be hoped will speedily supersede the amorphous and unreliable preparations of very variable potency now met with commercially under the name of (so-called) "aconitine."

The following numbers were obtained with these four specimens, A, B, C, and D. In each case the corrected melting point was almost exactly the same as that previously found with the other specimens; A and C melted at 185.5° , B at 183° , and D at 181° : the small quantity of specimen D obtained, and the consequent difficulty in thoroughly purifying is evidently the cause of the somewhat low melting point found.

- (A.) 0.2640 gram gave 0.6165 CO₂, and 0.1790 H₂O.
 0.4090 „ burnt with soda lime gave by titration 0.00938
 nitrogen; by conversion into Pt salt 0.0605 Pt.
 0.7170 gram gold salt gave 0.1495 Au.
 (B.) 0.3325 „ gave 0.7750 CO₂, and 0.2250 H₂O.
 (C.) 0.3700 „ gave 0.8620 CO₂, and 0.2550 H₂O.
 0.6420 „ gold salt gave 0.1320 Au.
 (D.) 0.3675 „ gave 0.8570 CO₂, and 0.2540 H₂O.

	Carbon in base.	Hydrogen.	Nitrogen.	Gold in gold salt.
(A)	63.69	7.53	$\left\{ \begin{array}{l} 2.29 \\ 2.09 \end{array} \right.$	20.85
(B)	63.57	7.52	—	—
(C)	63.54	7.66	—	20.56
(D) ..	63.60	7.68	—	—
Average of 7 speci- mens examined } previously }	63.58	7.50	2.27	20.43
Calculated for C ₆₆ H ₈₈ N ₂ O ₂₁ }	63.67	7.07	2.25	20.39

It is hence evident, firstly, that only one crystallisable alkaloid was contained in this third batch of roots; secondly, that this alkaloid was identical with that contained in the two batches of root previously examined, and thirdly, that precisely the same substance resulted whether the alcohol employed were acidulated with tartaric acid or not: in other words, that if, as is probable, the roots originally con-

tain an alkaloid $C_{33}H_{47}NO_{12} = (C_{26}H_{39}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ = OH \\ \diagdown OH \end{array}$, this becomes

dehydrated to the sesqui-apo-derivative termed above japaconitine, and indicated by the formula—

by the heat alone employed in evaporating off the alcohol, even if no tartaric acid at all be employed to acidulate the alcohol.

The non-crystalline bases accumulating in the ethereal mother-liquors of the first rough crystals of japaconitine obtained from this batch were examined with the following results. Carbon = 65.73, hydrogen = 8.07, nitrogen = 2.69 per cent.: benzoic acid formed on saponification with alcoholic potash = 19.1 per cent. After saponi-

fication, the base produced was almost wholly dissolved by water, and closely resembled aconine. From these results, it seems to follow that the amorphous base was almost wholly of the saponifiable class, and closely related to japaconitine; in which respect it seems to differ somewhat from the amorphous bases obtained from *A. Napellus* (Parts I and III), which yielded much less benzoic acid on saponification, and therefore presumably contained a large admixture of a non-saponifiable alkaloid.

In conclusion, the following deductions may be drawn from the preceding experiments:—

(1.) The aconite roots imported from Japan appears to be tolerably uniform in character, and considerably richer in active crystallisable alkaloids, as well as in the non-crystalline bases, than *A. Napellus* root.

(2.) The active crystallisable alkaloid present is, as supposed by Paul and Kingzett, different from both aconitine and pseudaconitine, although closely allied to both, and especially to the former.

(3.) Only one crystallisable alkaloid could be isolated from each of three different batches of roots examined; this alkaloid is indicated by the formula $C_{86}H_{88}N_2O_{21}$ (and not by the approximate formula attributed by Paul and Kingzett, $C_{29}H_{43}NO_9$).

(4.) This alkaloid, to which it is proposed to give the provisional name *japaconitine*, breaks up on saponification into benzoic acid, and a new base *japaconine*, $C_{26}H_{41}NO_{10}$; on treatment with benzoic anhydride, it forms a derivative containing four benzoyl groups for every C_{33} originally present, in this respect differing from aconitine, which only forms a dibenzoylated derivative on similar treatment. The same derivative is formed on benzoylating japaconine. Japaconitine and japaconine very closely resemble aconitine and aconine respectively in all their physical properties, so that save in analytical numbers and by benzoylating, their discrimination is almost impracticable.

(5.) Contrary to the statements of Paul and Kingzett, japaconitine readily forms crystallisable salts, especially with nitric, hydrochloric, and hydrobromic acids; the hydrobromide, like that of aconitine, contains $2\frac{1}{2}$ H_2O for every C_{33} present.

(6.) In isolating japaconitine from the roots, plain alcohol may be used instead of alcohol acidulated with tartaric acid (or with a mineral acid); practically all the alkaloid present is thus easily extracted. Probably the same remarks apply to other kinds of aconite roots, wherefore in all probability it is better in such cases to use plain alcohol only for the purpose of extracting the alkaloids.

(7.) The relationships of japaconitine to its derivatives and to aconitine are conveniently expressed by regarding japaconitine as being formed by the sesqui-dehydration of an alkaloid, $C_{33}H_{47}NO_{12}$

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(not yet isolated in an unaltered state), closely related to aconitine, as indicated by the following table:—

<i>Japaconitine Series.</i>	<i>Aconitine Series.</i>
Hypothetical parent base, $(C_{26}H_{39}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ -O.H \\ -O.H \\ \diagdown O.H \end{array} .$	Aconitine, $(C_{26}H_{35}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ -O.H \\ -O.H \\ \diagdown O.H \end{array} .$
Sesqui-apo-derivative (japaconitine), $(C_{26}H_{39}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ \equiv O \\ \\ O \\ \\ (C_{26}H_{39}NO_7) \begin{array}{l} \diagup O \\ \equiv O \\ \diagdown O.C_7H_5O \end{array} \end{array} .$	Apo-derivative (apo-aconitine), $(C_{26}H_{35}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ \equiv O \\ \diagdown OH \end{array} .$
Benzoyl-derivative (tetrabenzoylated), $(C_{26}H_{39}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ -O.C_7H_5O \\ -O.C_7H_5O \\ \diagdown O.C_7H_5O \end{array} .$	Benzoyl-derivative (dibenzoylated), $(C_{26}H_{35}NO_7) \begin{array}{l} \diagup O.C_7H_5O \\ \equiv O \\ \diagdown O.C_7H_5O \end{array} .$
Saponification product (japaconine), $(C_{26}H_{39}NO_7) \begin{array}{l} \diagup OH \\ \equiv O \\ \diagdown OH \end{array} .$	Saponification product (aconine), $(C_{26}H_{35}NO_7) \begin{array}{l} \diagup OH \\ -OH \\ -OH \\ \diagdown OH \end{array} .$
	Apo-aconine, $(C_{26}H_{35}NO_7) \begin{array}{l} \diagup OH \\ \equiv O \\ \diagdown OH \end{array} .$