305. *Curare Alkaloids. Part III. Pot-curare.*

By Harold King.

The curare arrow-poisons have been frequently tried in medicine. The object of the present investigation was the isolation of the alkaloidal active principle, or other constituent alkaloids of pot-curare, in the hope that the nature of the active principle and its botanical origin might be revealed. It has been found that the specimen of pot-curare under examination contains many phenolic alkaloids, two of which, neoprotocuridine and protocuridine, have now been well characterised. They are isomeric, each with the formula $C_{36}H_{38}O_6N_2$, and on exhaustive methylation give isomeric *O*-methyl methiodides $C_{40}H_{48}O_6N_2I_2$. Neoprotocuridine has been identified as an internally compensated form of *iso*chondrodendrine, an alkaloid of *Radix Pareiræ bravæ* originating from *Chondrodendron tomentosum* R. and P. This establishes a close relationship between the alkaloids of pot-curare and those of tube-curare. Both are based on the fusion of two polyphenolic benzyl*iso*quinoline nuclei by ether linkages. The presence of other alkaloids in pot-curare has been established by complete methylation. The active principle has been isolated as an amorphous phenolic quaternary iodide of high paralysing activity. The botanical origin is discussed in terms of the observations of explorers and botanists and the conclusion is reached that the alkaloids of this specimen of pot-curare originated from an Amazonian plant of the N.O. *Menispermaceæ* without the addition of any *Strychnos* species.

Pot-curare is the name given to the South American arrow-poisons put up in earthenware pots. These containers are of various sizes and may be unglazed, glazed or ornamented with paint; they are characteristic of the curare prepared by the Indian tribes who dwell on the borders of Brazil, Peru, and Ecuador, round the waters of the Upper Amazon.

Although curare has been the subject of chemical enquiry for over a hundred years, there appear to be only two clear records of the examination of curare from pots. In 1862 Buchner (*Arch. Pharm.*, **160**, 19) examined a pot-curare brought by the German botanist and explorer von Martius from the Upper Amazon. He was unable to crystallise the active principle, but found that it was bitter, alcohol-soluble, and gave a colour reaction not unlike that of strychnine. In the last few years of the 19th century, Boehm (*Abhandl. Kgl. sächs. Ges. Wiss.*, 1897, **24**, 22) brought order into the curare field by showing that the type of container was in general diagnostic of the type of curare contained therein. From various sources he collected a number of pots of curare which yielded 250 g. of crude drug but of widely differing physiological activity. This material was only sufficient to give Boehm a general orientation of the problem of pot-curare and to determine the method by which, in his opinion, the material should be worked up. Pot-curare was found to differ chemically from calabash- or gourd-curare in that an aqueous extract of the former gave a voluminous precipitate with metaphosphoric acid and a precipitate with ammonia. In this respect it resembled tube-curare, but unlike the latter, its examination presented great experimental difficulties. Only one specimen of pot-curare gave a "curarine," *i.e.*, a quaternary alkaloid

with intense curare activity. This amorphous alkaloid was called protocurarine; it gave a colour reaction (sulphuric acid and dichromate) reminiscent of strychnine. The non-quaternary alkaloids, however, called in general "curines," proved to be present as a class in all the specimens of pot-curare. From the non-quaternary alkaloids Boehm isolated a small quantity of two crystalline bases, protocurine, m. p. 306°, and protocuridine, m. p. 274—276°. They were characterised by their sparing solubility in organic solvents, and by forming crystalline sulphates and platinum salts. The latter were analysed for carbon, hydrogen, and platinum only, so the formulæ $C_{20}H_{23}O_3N$ and $C_{19}H_{21}O_3N$ attributed to the two alkaloids respectively were, in the absence of nitrogen analyses, only tentative. The two bases showed no characteristic colour reactions, but protocurine had a weak curare action on frogs.

Through the generosity of Sir Charles Sherrington, O.M., the author acquired two specimens of pot-curare in their original containers, which were given to him by the widow of the late Professor A. A. Kanthack. The pots probably came from the Amazon region, since Kanthack was born at Bahia, Brazil, and his father was Consul at Para at the mouth of the Amazon. The smaller of the two pots, a prior claim to which was very kindly relinquished by Professor Robert Robinson, is the subject of the present communication. It is an unglazed pot (diameter 7·5 cm., height 5 cm.) and contained about 30 g. of undisturbed pot-curare. Its curare activity was not very intense, since the paralysing dose (see below) was about 27 mg. per kg. of frog. Lapicque and Veil (*Compt. rend. Soc. Biol.*, 1928, **99**, 488) examined two pot-curares and found paralysing doses of 10 and 12 mg. per kg. of frog, whilst Santesson (*Acta Med. Skand.*, 1931, **75**, 7; *Skand. Arch. Physiol.*, 1936, **74**, 142) recorded two pot-curares of the same order of activity. The larger of the two pots presented by Sir Charles Sherrington contained a pot-curare far more active than any of these, as the paralysing dose was 1 mg. per kg. of frog. It has not yet been subjected to chemical investigation.

Preliminary experiments showed that in pot-curare one was dealing with a difficult alkaloidal mixture of unusual properties. By applying the methods which the author has found successful in the examination of tube- and calabash-curare and of many natural barks, it was found that pot-curare contained a mixture of many alkaloids, entirely phenolic, which, owing to the insolubility of the bulk of them, could not be taken up in chloroform or ether. This rendered the quantitative separation of quaternary from non-quaternary alkaloids impossible. In addition, saponin-like substances were present which gave rise to stable emulsions and to solutions which foamed intensely.

The method finally adopted was, in brief, to extract all soluble material by means of 1% tartaric acid solution at 100°, to precipitate complex non-alkaloidal impurities and tartaric acid with basic lead acetate, and then to precipitate the main bulk of non-quaternary alkaloids from concentrated solution with saturated sodium bicarbonate solution. The "non-quaternary" bases so obtained constituted 38% of the original curare, whilst the so-called "quaternary fraction" contained another 12% of alkaloidal bases. The original pot-curare thus contained half its weight of alkaloidal bases.

As the kernel of interest in pot-curare lies in its paralysing principle, all chemical operations have been followed by a determination of the paralysing dose on frogs under the standard conditions later defined (p. 1477). Using this frog test, it was found that the non-quaternary bases showed a weak curare action, but most of the activity was in the "quaternary fraction;" at the same time, some loss of activity was apt to occur through adsorption on precipitates and to be non-recoverable by hot solvents.

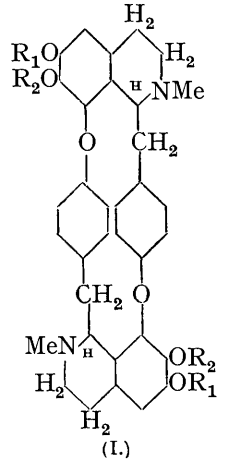
On submitting the amorphous non-quaternary fraction of the dried bases to continuous ether extraction for many weeks, a partly crystalline mixture of bases separated in the ethereal extract, which on solution in *N*-sodium hydroxide slowly deposited the crystalline sodium salt of an alkaloid, for which the name *neoprotocuridine* is proposed. By saturating the alkaline mother-liquor with carbon dioxide in the presence of chloroform, the whole of the residual phenolic bases was transferred to chloroform. On concentration of this solvent, a mixture of sparingly soluble crystalline alkaloids was obtained which, by crystallisation as the hydrochlorides, could be separated almost completely into the sparingly soluble *dihydrochloride* of neoprotocuridine and the much more readily soluble hydrochloride

of the alkaloid protocuridine discovered by Boehm. When pure neoprotocuridine base was liberated from a solution of its hydrochloride it could not be extracted by chloroform, but it slowly crystallised from the aqueous phase and could then be recrystallised from water. It had m. p. 232° and formed a well-crystallised sulphate, which, being readily water-soluble, was suitable for the determination of the specific rotation. It proved to be optically inactive. The most characteristic reaction of neoprotocuridine was its behaviour towards nitric acid. A crystalline nitrate was formed in very high dilution, but nitric acid solutions of the base could not be heated, since rapid oxidation of the alkaloidal base occurred even at 75°. Neoprotocuridine had a weak curare action.

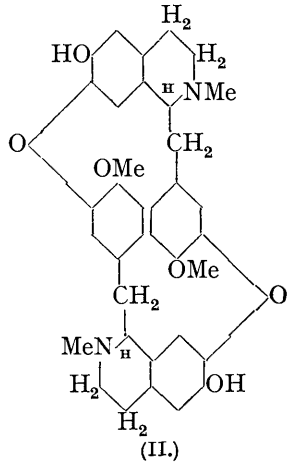
On the basis of the micro-analysis of neoprotocuridine, its hydrochloride, and its methylation product O-*methylneoprotocuridine methiodide*, there is little doubt that neoprotocuridine has the formula $C_{36}H_{38}O_6N_2,8H_2O$. The dihydrochloride is $C_{36}H_{38}O_6N_2,2HCl$, 6 or 7 H_2O, whilst methylneoprotocuridine methiodide has the formula $C_{40}H_{48}O_6N_2I_2$. The parent base neoprotocuridine has two phenolic groups and two methoxy-groups, the two former becoming methylated in methylneoprotocuridine methiodide which is no longer phenolic. The two remaining oxygen atoms are probably present as ether linkages.

Protocuridine hydrochloride has the formula $C_{36}H_{38}O_6N_2,2HCl,6H_2O$, and on complete methylation gave crystalline O-*methylprotocuridine methiodide* $C_{40}H_{48}O_6N_2I_2$ containing 4 methoxy-groups. The parent base protocuridine was obtained by Boehm as a crystalline powder, m. p. 280°, which was insoluble in all solvents. It has now been found that it can be crystallised from aqueous pyridine and then melts at 295°. A satisfactory analysis of the base was not obtained, since pyridine cannot be completely removed, a property also observed by Scholtz for the phenolic alkaloid *iso*bebeeridine (*Arch. Pharm.*, 1915, **253**, 622). The most characteristic reaction of protocuridine is the Millon reaction; this is not shown by neoprotocuridine. Furthermore, protocuridine hydrochloride, unlike neoprotocuridine, is optically active, $[\alpha]_{5461} + 7{\cdot}6°$.

Protocuridine and neoprotocuridine are therefore two diphenolic isomeric bases of the formula $C_{36}H_{38}O_6N_2$, and on complete methylation give two different *O*-methyl methiodides, $C_{40}H_{48}O_6N_2I_2$. It is significant that the latter formula is the same as that found by the author for *O*-methyltubocurarine iodide (J., 1935, 1381), the *O*-methylation product of the active principle of tubocurare, whilst the formulæ for the parent bases show that they are isomeric with bebeerine, *iso*bebeerine (chondrodendrine, *iso*chondrodendrine), and other allied substances. The unusual properties, moreover, of protocuridine and neoprotocuridine necessitate a high molecular weight. The author is tempted to suggest that these two bases are, in fact, two further members of the bisbenzyl*iso*quinoline group of alkaloids formed by fusion of two norcoclaurine units (King, *Ann. Reports*, 1933, **30**, 242; Kondo and Tomita, *Arch. Pharm.*, 1936, **274**, 65). Since neoprotocuridine is optically inactive, the most probable constitution (if it is not a racemate) is either (I) or (II), both of which have centrosymmetrical forms.



(I.)



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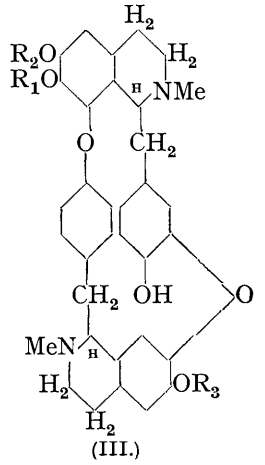

(III.)

In (I) if R_1 = H then R_2 = Me or *vice versa*. In (II) an alternative arrangement of methoxyl and phenolic groups is not possible, since neoprotocuridine does not give a Millon reaction. In structures of these types this colour reaction seems to be shown typically only by phenolic groups in the positions occupied by the methoxy-groups in the benzyl radicals in formula (II) (see Experimental). In passing, it may be mentioned that bebeerine and tubocurarine chloride show the Millon reaction, an observation which will be of service in determining the still-unknown relative orientation of phenolic and methoxyl groups in these alkaloids. For neoprotocuridine, (I) is the more probable structure; on this basis neoprotocuridine should be a stereoisomeride of *iso*bebeerine (*iso*chondrodendrine) and both on complete methylation and Hofmann degradation should give the same optically inactive methine. On putting this assumption to the test, it was found that neoprotocuridine gave a crystalline methine or mixture of methines which on further methylation gave a methylneoprotocuridinemethine methiodide, unmelted at 320° and indistinguishable from inactive α-methyl*iso*chondrodendrinemethine methiodide, prepared from a specimen of inactive α-methyl*iso*chondrodendrinemethine kindly supplied by Professor Faltis of Vienna. Comparison of the two was not possible by the melting points but was facilitated by the observation that both give a cherry-red colour in sulphuric acid which changes on warming into an indigo-blue. This reaction appears to be highly specific, since it is not given by the isomeric optically active β-methyl*iso*chondrodendrinemethine methiodide or by any of the four isomeric methine methiodides obtained from tubocurarine or bebeerine (King, J., 1935, 1381). Neoprotocuridine must therefore have the structure shown in (I), where R_1 and R_2 are hydroxyl and methoxyl respectively or *vice versa*.

Since protocuridine is optically active and gives a typical Millon reaction, it cannot have any structure based on (I). A structure based on (II) is only possible when there is an interchange of one or both of the methoxyl groups in the benzyl radicals with the phenolic groups in the *iso*quinoline nuclei. If both central groups are phenolic, then the non-centrosymmetrical stereoisomeride must be chosen so as to confer optical activity. The possibility seems to be excluded that protocuridine might be based on a hybrid between (I) and (II) such as (III) where one of the radicals R_1, R_2, R_3 is hydrogen and the other two methyls. This is the nuclear structure of bebeerine and tubocurarine chloride, and completely methylated protocuridine is different from the only two diastereoisomerides *O*-methylbebeerine methiodide and *O*-methyltubocurarine iodide which are possible for this structure, unless indeed an obstacle effect of the central benzene nuclei is playing a part in giving rise to an increased number of stereoisomerides (cf. Faltis, Wrann, and Kühas, *Annalen*, 1932, **497**, 69).

We turn now to the so-called " quaternary fraction," which showed the more intense curare action. This fraction, after removal of non-basic material by phosphotungstic acid precipitation, was crystallised as chloride, and readily gave sparingly soluble neoprotocuridine hydrochloride, identical with the salt obtained from the non-quaternary fraction. After removal of this salt and suitable concentration, the " quaternary fraction " was found to be precipitable by saturated sodium bicarbonate solution to the extent of 50%. The precipitate was, however, water-soluble, and consisted either of a mixture of phenolic betaines or of complex alkaloidal salts salted out by the bicarbonate. The precipitate had a paralysing dose of about 15 mg. per kg. of frog and has not been examined further. The non-precipitable " quaternary " alkaloids, having approximately twice the activity, were submitted to fractional precipitation with mercuric chloride, 11 fractions being collected. When assayed by the frog test, the activity was found to be fairly uniformly distributed and to be completely precipitable by mercuric chloride. Fraction 9 (80 mg.) had the highest activity, the paralysing dose being 4 mg. per kg. of frog. It was fractionally precipitated from methyl-alcoholic solution by ether, but in none of the six fractions collected was there any outstanding concentration of activity. These six fractions were recombined and precipitated in concentrated aqueous solution by addition of solid sodium bicarbonate; the precipitate had an activity of 10 mg. per kg. The mother-liquor was neutralised with hydriodic acid, and deposited an amorphous iodide (33·5 mg.), with a paralysing dose of 1·5 mg. per kg. of frog, *i.e.*, one-third the activity of tubocurarine chloride. This is the most active material obtained in this investigation. This iodide is phenolic and gives a

Millon reaction, but the proof that the iodide is that of a quaternary base has not been established beyond doubt. The product does not, however, show the strychnine-like colour reaction with dichromate and sulphuric acid, and it can have no connection with the protocurarine of Boehm.

All attempts to crystallise any of the fractions from the mercury precipitation of the main " quaternary " fraction with various acids failed, but that a mixture of alkaloids was present was shown by exhaustive methylation. Thus, methylation of fraction 8 with methyl iodide and methyl-alcoholic potash gave a very sparingly soluble microcrystalline alkaloidal *methiodide A*, of the unusual empirical composition $C_{20}H_{25}O_8NI_2$. It was almost insoluble in boiling organic solvents, and in boiling water its solubility was less than 1 part in 5000.

Similarly, by methylation of a combination of 7 other fractions from the mercury precipitations, a much more soluble alkaloidal *methiodide B* has been obtained, crystallising in fine needles, m. p. 318°. In crystalline properties and m. p. it agreed with methylprotocuraridine methiodide, but identity cannot at present be assumed on account of the analytical results. In addition, a minute quantity of a very sparingly soluble alkaloidal methiodide C has been obtained, unmelted at 295° and differing from any previously described.

The Botanical Origin of Pot-curare.

On the evidence of explorers and botanists, it is certain that pot-curare is made by Indian tribes of the Upper Amazon in Brazil. De la Condamine (*Hist. Acad. Sci.*, 1745, Paris 1749; *Mém.*, 489) reported that the arrow poison prepared by the Ticuna Indians was the best obtainable along the whole length of the Amazon, and that the Ticunas used the juice of many plants, especially lianes. von Martius (*Buchner's Repert. Pharm.*, 1830, **36**, 337) saw the preparation of pot-curare (urari) by the Juris on the Rio Yupurá in the Upper Amazon, and identified the chief plant used as *Rouhamon guianensis* Aubl. [now called *Strychnos guianensis* (Aubl.) Baill.]. In addition, among other plants used, there was a hitherto undescribed species, *Cocculus Imene* Mart. [= *Abuta Imene* (Mart.) Eichl.], which was probably replaced occasionally by *Cocculus grandifolius* Mart. von Spix, who accompanied von Martius on his travels, brought back a liane which was used by the Ticuna Indians and this was considered to be probably a *Cocculus* by von Martius, who called it *Cocculus amazonum*. Some years later, de Castelnau and the botanist Weddell (*Expédition dans Le Brasil et Le Perou*, 1851, **5**, 21) also saw curare prepared by the Ticuna Indians and neighbouring tribes. Weddell identified the plants used and named them *Strychnos Castelnaei* Wedd. (= *St. Castelnaeana* Baill.) and *Cocculus toxicoferus* * Wedd. They noticed that the quantity of the *Cocculus* used exceeded that of the *Strychnos*, since the former was a commoner plant. The explorer Jobert (*Compt. rend.*, 1878, **86**, 121; 1879, **89**, 646) speaks frequently of the adulteration of curare, and mentions that the Pebas prepared a curare which contained little or no *Strychnos* ingredients, but mainly a plant of the N.O. *Menispermaceæ*. The botanist Schwacke (*Jahrb. Kgl. bot. Gart. bot. Mus.*, Berlin, 1881—4, III, 220), who accompanied Jobert and assisted in the preparation of Ticuna curare, describes two of the ingredients as *Strychnos Castelnaei* Wedd. and *Anomospermum grandifolium* Eichl. [now known as *Elissarrhena grandifolia* (Eichl.) Diels].

The last-named species was examined by de Lacerda (*Arch. Mus. Nac.*, Rio de Janeiro, 1901, **11**, 163) who found that a crude extract had a true curare action. Unfortunately, no quantitative data are given. de Lacerda made the suggestion that the *Cocculus amazonum* of von Martius might be the same species as *Anomospermum grandifolium* of Eichler. Cl. Bernard, however, made a decoction of *Cocculus amazonum* and failed to find a curare action. de Lacerda's general conclusion was that the principal plant used in the preparation of pot-curare is a *Menisperm* and not a *Strychnos*.

* The exact identity of *Cocculus toxicoferus* and *amazonum* is unknown. The genus is uncertain owing to lack of flowers and fruit for identification, but there is no doubt about their inclusion in the N.O. *Menispermaceæ*. Maheu (Rech. Anatom. sur les Menispermacées, *J. Bot.*, Paris, 1902, **16**, 369) was of the opinion that *Cocculus toxicoferus* of Weddell is not a *Cocculus* but should be put into the monotypic genus *Strychnopsis* of Baillon endemic in Madagascar.

The French explorer Crevaux (*Compt. rend.*, 1879, **89**, 1023) brought back all the plants used on the Upper Amazon in the preparation of curare; from *Strychnos Castelnaei* Wedd. he prepared a curare ten times as strong as the Indian preparation. Rodriques ("Curare," Bruxelles, 1903), however, was of the opinion that curare made from plants of the N.O. *Menispermaceæ* (this includes the sub-families *Cocculus*, *Anomospermum*, *Chondrodendron*, *Abuta*, etc.) was destined for commerce or exchange with other tribes.

From the foregoing historical survey there are strong indications that the essential ingredients of pot-curare are prepared either from a *Strychnos* species with the addition of a *Menisperm* or from a *Menisperm* alone. The small pot of pot-curare which is the subject of this communication clearly belongs to the latter category. All the alkaloids are phenolic, and none gives a colour reaction reminiscent of strychnine, whilst the composition and characters of the crystalline alkaloids neoprotocuridine and protocuridine and their formulation as bisnorcoclaurine alkaloids are in agreement with their origin from a *Menisperm*. The identification of neoprotocuridine as an internally compensated centro-symmetrical form of *iso*chondrodendrine brings this type of pot-curare into close relationship with tube-curare which is made on the upper waters of the Amazon in Peru (River Ucayali district) and contains two closely related alkaloids, *l*-curine (= *l*-bebeerine or α-chondrodendrine) and *d*-tubocurarine chloride (King, *loc. cit.*). The latter had an activity of 0·5 mg. per kg. of frog, *i.e.*, about three times that of the amorphous iodide isolated in this investigation. The possibility that this active principle (or principles) contains tubocurarine is not excluded, but in any case the properties of the most active fraction are consistent with its being a quaternary alkaloid derived from a partly methylated bisnorcoclaurine base. It is noteworthy that the bisnorcoclaurine alkaloids of the S. American Menisperms are based, so far as is at present known, exclusively on a centro-symmetrical juxtaposition of two benzyl*iso*quinolines, whereas the Asiatic Menisperms have only yielded bisnorcoclaurine alkaloids based on a plano-symmetrical juxtaposition of benzyl*iso*quinolines.

The evidence of the botanist Schwacke (*loc. cit.*) that two of the species used by the Ticuna Indians are *Strychnos Castelnaei* Wedd. and *Anomospermum grandifolium* Eichl. is corroborated by de Lacerda's preparation of an active extract with a true curare action from *Anomospermum grandifolium* Eichl. and by Crevaux's preparation of a strong curare from *St. Castelnaei* Wedd. de Lacerda's suggestion, however, of possible identity of *Cocculus amazonum* Mart. with *Anomospermum grandifolium* Eichl. is not supported by Cl. Bernard's experimental results. It is therefore clearly desirable that chemical examination of the Menisperm, *Anomospermum grandifolium* Eichl., should be undertaken, for this may be the key to the phenolic alkaloids of pot-curare.

In conclusion it is necessary to refer to the two *Strychnos* species, *St. guianensis* (Aubl.) Baill. and *St. Castelnaei* Wedd., which have been identified as being used in the preparation of some specimens of pot-curare. Through the valuable co-operation of Mr. B. N. Wood, the Curator of Forests, British Guiana, and the Senior Forestry Officer, Mr. T. A. W. Davis, I have been able to examine the bark of a liane which was identified by Mr. N. Y. Sandwith of the Royal Botanic Gardens, Kew, as "near *St. guianensis* (Aubl.) Baill. and possibly a form or variety." A concentrated extract of this bark was devoid of curare action on frogs and contained little or no alkaloid. This receives support from Robert Schomburgk's statement ("Reisen in Guiana und am Orinoco," 1835—39, Leipzig, 1841, 94) that the Indian tribes who make curare from *St. guianensis* Aubl. prefer that made from *St. toxifera* Schomb. as being more active. Again, through the kindness of Mr. Gruber and Dr. R. T. Major, of the firm of Merck and Co. Inc. of Rahway, N.J., I was able to examine a small quantity of the bark of *St. Castelnaei* Wedd. This contained about 0·2% of amorphous non-quaternary alkaloid and a like quantity of amorphous quaternary alkaloid. The latter gave a strychinne-like colour reaction with dichromate and sulphuric acid and had a strong curare action (1·5 mg. per kg. of frog). The alkaloids were non-phenolic and bore no resemblance to those encountered in this investigation on pot-curare.

Experimental.

The Frog Test for Curare.—For the determination of curare activity it is essential to have a quick and reliable roughly quantitative test. Professor J. H. Gaddum, who as a former member

of our staff carried out the preliminary tests on curare, defined the paralysing dose for frogs as that quantity of curare which paralyses the righting reflex within 15 minutes, as shown by the inability of the animal when laid on its back to recover its normal posture within 1 minute, the volume of solution injected into the ventral lymph sac being 0·02 c.c. per g. of frog. The true curare nature of the paralysis was frequently checked by an observation of the effect of electrical stimulation of the exposed sciatic nerve and gastrocnemius muscle of the pithed animal. The later tests were carried out by Dr. G. L. Brown and by Miss W. I. Strangeways, and I desire to acknowledge the cordial co-operation of these three workers, without whose aid the results of the investigation could not have been attained.

Pot-curare.—25 G. of pot-curare were heated for a short time with 1% tartaric acid solution (750 c.c.) and sufficient 50% solution (5 c.c.) to make the solution faintly acid to Congo-red paper. After filtration, the undissolved solid was re-extracted with a further 100 c.c. of 1% tartaric acid. The combined filtrates (920 c.c.) were precipitated with basic lead acetate solution (200 c.c.), the precipitate removed and washed. The precipitate and the filtrate A were separately decomposed with hydrogen sulphide. The aqueous filtrate obtained on decomposing the lead precipitate was devoid of activity on the frog and was discarded. The original extract (920 c.c.) had an activity corresponding to 27 mg. of original drug per kg. of frog. An earlier assay on a separate preparation gave 29 mg. per kg. of frog.

The filtrate A was concentrated and treated at 0° with saturated sodium bicarbonate solution (170 c.c.) so long as a precipitate was formed. After 12 hours' keeping at 0° the precipitate B was collected; yield 8·65 g. The bicarbonate-alkaline filtrate was extracted, once each, with ether and chloroform, further extraction being impossible owing to the extremely difficult emulsions which were formed and which could only be temporarily resolved by filtration through kieselguhr. The chloroform and ethereal extracts were evaporated; the residue was dissolved in *N*-hydrochloric acid which had been used for extraction of alkaloidal bases from the kieselguhr, and after concentration to a small volume was precipitated by addition of solid sodium bicarbonate. The precipitated base (1·0 g.) was added to the main bulk of non-quaternary alkaloids, B. The curare activity of this non-quaternary fraction was relatively weak, 192 mg. per kg. of frog.

Examination of Non-quaternary Fraction B.

The crude dried bases, 9·65 g., were submitted to continuous extraction (Soxhlet) with dry ether for many weeks. As extraction proceeded, partly crystalline bases separated in the ethereal extract. The extraction flask was changed at long intervals, the total solid obtained from the ethereal extracts being 4·85 g. The unextracted alkaloids, 4·8 g., have been set aside for future examination.

After many preliminary experiments the following method gave satisfactory results for isolating two crystalline alkaloids from the ether-soluble bases. A portion of these bases, 850 mg., was dissolved in *N*-sodium hydroxide (7·5 c.c.) and kept at 0° for 36 hours. A crop of microscopic short needles of the sodium salt of neoprotocuridine (89·7 mg.) which separated was collected and washed with *N*-sodium hydroxide. The filtrate was saturated with carbon dioxide in the presence of a liberal quantity of chloroform, and the alkaloids completely removed by 6 extractions. The combined chloroform extract was concentrated to a small volume and readily deposited small plates as the solution boiled down. This crystalline mixture of protocuridine and neoprotocuridine (102 mg.) had m. p. 284°. It apparently contained chloroform of crystallisation, since it rapidly lost over 10% in weight when exposed to the air for a few hours. The remaining alkaloids not crystallisable as a sodium salt or from chloroform have been set aside for future examination.

Sodium neoprotocuridine (143 mg.) dissolved in boiling 0·25*N*-hydrochloric acid (35 c.c.) deposited on keeping a microcrystalline powder (91 mg.) of neoprotocuridine hydrochloride, unmelted at 310°, giving no Millon reaction but yielding a characteristic nitrate in high dilutions. If the hydrochloride is dissolved in a little hot water, addition of 2*N*-sodium hydroxide solution soon reproduces sodium neoprotocuridine in microscopic plates. Fuller details of neoprotocuridine are given under the section dealing with the " quaternary fraction."

Separation of Neoprotocuridine and Protocuridine.—The amount of these bases available from the chloroform crystallisation experiment was 506 mg. This was dissolved in 0·5*N*-hydrochloric acid and fractionally crystallised. Neoprotocuridine hydrochloride, being very sparingly soluble and separating as a rule as a microcrystalline powder, was readily obtained, and from the mother-liquors *protocuridine hydrochloride* (236 mg.), octahedra, m. p. 295° (efferv.) (Found: loss at 100° in a high vacuum, 13·1. $C_{36}H_{38}O_6N_2,2HCl,6H_2O$ requires H_2O, 13·9%. Found, for anhydrous salt: C, 64·3; H, 6·2; N, 4·5; Cl, 9·9. $C_{36}H_{38}O_6N_2,2HCl$ requires C, 64·7; H, 6·0; N,

4·2; Cl, 10·6%). The specific rotation of the anydrous salt was determined in water: $[\alpha]_{5461}^{20°}$ + 7·6° (c = 0·3). *Protocuridine* base was precipitated as an amorphous solid on addition of sodium bicarbonate to a solution of the hydrochloride, but, on being kept in contact with the solution, it changed into compact tablets. It is not soluble in 2*N*-sodium hydroxide solution (formation of an insoluble salt) but dissolves immediately on dilution. It gives a strong Millon reaction but no coloration with ferric chloride. The only solvent from which it could be recrystallised was aqueous pyridine. The solid was dissolved in pure boiling pyridine, and water added dropwise until crystallisation set in. The base separated in irregular shaped plates, m. p. 295° (Found: loss at 100° in a high vacuum, 11·3. Found in dried material: C, 72·2; H, 6·5; N, 5·7. $C_{36}H_{38}O_6N_2,\frac{1}{2}C_5H_5N$ requires C, 73·2; H, 6·4; N, 5·5%). The sulphate crystallises readily in pointed tablets, and the platinichloride in compact anisotropic tablets. The last two salts are in substantial agreement with Boehm's observations. O-*Methylprotocuridine methiodide* was prepared by boiling protocuridine hydrochloride (76·2 mg.) with 0·5*N*-methyl-alcoholic potassium hydroxide (1 c.c.) and methyl iodide (0·5 c.c.), a second portion of the methylating agents being added after 30 minutes. The neutral solution deposited straw-coloured needles (81·8 mg.), which were crystallised from methyl alcohol (4 c.c.) and then separated as cream-coloured needles, m. p. 318° (decomp.) (Found: loss at 100° in a high vacuum, 2·1. Found, in dried material: C, 52·9, 52·6; H, 5·6, 5·4; N, 3·1, 3·1; I, 27·0, 27·2; MeO 12·1, 11·8. $C_{40}H_{48}O_6N_2I_2$ requires C, 53·0; H, 5·3; N, 3·1; I, 28·0; 4 MeO, 13·7%).

Examination of "Quaternary Fraction."

The mother-liquors (425 c.c.) from the sodium bicarbonate precipitation of non-quaternary bases, after neutralisation with sulphuric acid, were treated with pure sulphuric acid (21 g.), followed by 25% phosphotungstic acid solution (50 c.c.) in 5% sulphuric acid solution. The precipitate was collected, washed with 1% phosphotungstic acid solution in 5% sulphuric acid, and both the precipitate and the mother-liquor decomposed with baryta. The two filtrates were neutralised to Congo-red paper with dilute sulphuric acid, filtered from barium sulphate, and the two filtrates treated with sufficient barium chloride solution to remove sulphate ions. The filtrate from the phosphotungstic acid precipitation was devoid of any frog-paralysing principle and was rejected. The precipitate which contained all the bases, now present as soluble chlorides, was concentrated at 50° under diminished pressure and finally over sulphuric acid in a vacuum. Three crops of a microcrystalline powder (1·0 g. in all) were collected, and on crystallisation from 30 volumes of boiling water gave pure *neoprotocuridine hydrochloride* (71 g.) [Found, in two different preparations: loss at 100° in a vacuum over P_2O_5; (*a*) 14·4, 14·8; (*b*) 16·7. $C_{36}H_{38}O_6N_2,2HCl,6H_2O$ and $7H_2O$ require H_2O, 13·9 and 15·9% respectively. Found, for anhydrous salt; (*a*) C, 65·1, 64·9; H, 7·0, 6·9; N, 4·4, 4·3; Cl, 9·6, 9·8; MeO, 5·0, 4·7. (*b*) C, 64·9, 65·0; H, 6·9, 7·0; N, 4·3, 4·3; Cl, 10·3, 10·0; MeO, 4·8, 5·1. $C_{36}H_{38}O_6N_2,2HCl$ requires C, 64·8; H, 6·0; N, 4·2; Cl, 10·6; 2MeO, 9·3%]. Although this hydrochloride usually separates as a microcrystalline powder composed of microscopic prisms, it can be obtained on very slow concentration of its solution in large prisms or plates. It is unmelted at 310° and does not give the Millon reaction. In very dilute solutions it gives a crystalline nitrate on addition of dilute nitric acid, and on warming the dilute nitric acid solution to 75° oxidation rapidly takes place. The salt swells up on addition of 2*N*-sodium hydroxide solution, forming a sodium salt which is soluble on dilution. However, on addition of ammonium chloride, the free base is not precipitated but the hydrochloride mixed with some base. Neoprotocuridine hydrochloride had a weak curare action, 45 mg. per kg. of frog producing paralysis in 40 minutes.

Neoprotocuridine.—The hydrochloride (162 mg.) in water (15 c.c.) was treated with saturated sodium bicarbonate solution (5 c.c.). The clear solution, which went yellow, was repeatedly extracted with chloroform, which did not, however, remove the base. When the aqueous solution was kept, the *base* crystallised out; yield 98 mg. This was dissolved in boiling water (4 c.c.), and separated in diamond-shaped leaflets, m. p. 232° with slight effervescence to a red melt (Found: loss at 110° in a high vacuum, 19·5. $C_{36}H_{38}O_6N_2,8H_2O$ requires H_2O, 19·5%. Found, in dried base: C, 72·0, 71·9; H, 6·7, 6·6; N, 4·7, 4·7; MeO, 9·2. $C_{36}H_{38}O_6N_2$ requires C, 72·7; H, 6·4; N, 4·7; 2MeO, 10·4%). The sulphate crystallises readily in rhomb-shaped leaflets, and, being much more soluble than the hydrochloride, was examined polarimetrically in solution (c = 0·8); it proved to be optically inactive. O-*Methylneoprotocuridine methiodide* was readily prepared by boiling neoprotocuridine hydrochloride (98 mg.) with 0·5*N*-methyl-alcoholic potash (2 c.c.) and methyl iodide (1 c.c.). A sparingly soluble potassium salt was first formed, complete methylation being attained by further addition of one-half the above quantities of methylating agents. The crystalline iodide (126 mg.) which separated was crystallised from boiling water

(30 c.c.) and separated in microscopic rhombs, unmelted at 300° (Found: loss in a high vacuum at 100°, 0·4. Found, in dried salt: C, 53·1, 53·0; H, 5·6, 5·5; N, 3·2, 3·1; I, 29·5, 29·8; MeO, 13·1, 12·8. $C_{40}H_{48}O_6N_2I_2$ requires C, 53·0; H, 5·3; N, 3·1; I, 28·0; 4MeO 13·7%).

Degradation of O-*Methylneoprotocuridine Methiodide.*—The iodide (0·49 g.) was converted into the chloride in warm aqueous suspension by digestion with excess of freshly precipitated silver chloride. The filtrate, when evaporated to a syrup, crystallised as a felt of needles of the corresponding chloride. It was boiled for 1·5 hours with 20% sodium hydroxide solution (20 c.c.), and then extracted thrice with ether and then thrice with chloroform. The former on evaporation left 0·2 g. of a clear gum, which readily crystallised in needles on addition of methyl alcohol (5 c.c.). The solution was then gently boiled with methyl iodide (1 c.c.) for an hour, whereupon a crystalline methiodide rapidly separated (yield 156 mg.). The chloroform extract was more pigmented and left a gum (0·1 g.) which was similarly methylated. A crystalline methiodide (143 mg.) also separated in this case. Both crops were carefully compared with an authentic specimen of α-*O*-methyl*iso*chondrodendrinemethine methiodide. Each salt was unmelted at 320°, and each on solution in sulphuric acid gave a cherry-red colour which became a purer red on warming and indigo-blue on stronger heating. Each specimen was sparingly soluble in boiling water and on rapid cooling gave a crystalline salt of identical appearance, compact spheres or clusters in which the shape of the individual crystals was not seen, but if the solutions were allowed to crystallise slowly, well-formed crystals separated of identical appearance in each case. Comparable solutions of all three salts in dilute solution gave a crystalline nitrate of the same characteristic appearance. The sulphates and hydrochlorides were soluble salts, but perchloric acid produced an amorphous precipitate from each.

The main alkaloidal "quaternary" liquor from which neoprotocuridine hydrochloride had been obtained was treated at 0° with saturated sodium bicarbonate solution (20 c.c.) so long as a precipitate was obtained. The friable precipitate was collected at 0° and on acquiring room temperature changed to a gum which was mainly water-soluble, was weakly alkaline, and was probably a mixture of phenolic betaines. With considerable difficulty, owing to emulsion formation, it was thoroughly extracted with chloroform, which removed a further quantity of non-quaternary alkaloids (0·3 g.). The neutralised extracted liquor when evaporated to dryness gave an amorphous residue (1·34 g.), which in a dose of 20 mg. per kg. of frog paralysed in 12 minutes. It has not been examined further.

The "quaternary fraction" still not precipitated by bicarbonate, after chloroform extraction which removed 0·1 g. of bases, was neutralised, and dried; yield 1·285 g. The paralysing dose was 8 mg. per kg. of frog in 12 minutes. This fraction, in water (10 c.c.), was stirred mechanically, and fractionally precipitated with powdered mercuric chloride, ten fractions being collected. Each was freed from mercury by treatment with hydrogen sulphide, but the first 8 fractions retained colloidal mercuric sulphide with persistence. By frog tests it was found that the whole of the active material had been precipitated and was fairly uniformly distributed throughout the fractions. Fraction 9, however (79·7 mg.), was slightly more active with a paralysing dose of 4 mg. per kg. within 11 minutes. It was further fractionally precipitated from methyl-alcoholic solution by dry ether, 6 fractions of approximately the same activity being obtained from which no crystalline salts could be obtained. The six fractions were recombined in a little water, and treated with solid sodium bicarbonate so long as a precipitate was formed. The precipitate (20 mg.) had a paralysing dose of 10 mg. in 13·5 minutes. The bicarbonate mother-liquor was neutralised with hydriodic acid solution, which caused an amorphous iodide (33·5 mg.) to separate. This had a paralysing dose of 1·5 mg. per kg. of frog in 15 minutes, *i.e.*, it had about one-third of the activity of *d*-tubocurarine chloride. The amount of material was insufficient for further fractionation. The iodide did not give a strychnine-like colour reaction with bichromate and sulphuric acid. It was instantly soluble in 2*N*-sodium hydroxide solution but not in sodium bicarbonate. It gave a Millon reaction but no sparingly soluble nitrate and it was not apparently sensitive to warm dilute nitric acid. It is therefore different from Boehm's protocurarine.

O-*Methylation of Fraction* 8.—All attempts to crystallise the water-soluble chlorides from the mercury fractionation as such or with other precipitants failed. Methylation was therefore resorted to in the hope of being able to obtain crystalline derivatives, although the active principle or principles present would at the same time become methylated and be irrecoverable. Fraction 8 (120 mg.) was gently boiled in 0·5*N*-methyl-alcoholic potassium hydroxide solution with methyl iodide. A heavy, white, microcrystalline solid (114 mg.) rapidly separated, which was almost insoluble in all solvents. It was possible, however, to recrystallise it by extracting it from a sintered-glass micro-extraction thimble with a small volume of boiling methyl alcohol;

yield 80·7 mg., m. p. 260° (decomp. after browning from 238°). This *iodide A*, was a whitish-cream powder showing anisotropic spheroidal crystals under the polarising microscope (Found: loss in a high vacuum at 100°, 1·1. Found, in dried material: C, 36·6, 36·5; H, 4·0, 4·0; N, 2·2, 2·1; I, 36·5, 37·3; MeO, 9·0, 9·1. $C_{20}H_{25}O_8NI_2$ requires C, 36·3; H, 3·8; N, 2·1; I, 38·4; 2MeO, 9·4%). The extreme insolubility of this substance probably necessitates a much higher molecular weight, possibly double that indicated. The high iodine and oxygen contents are unusual but are supported by duplicate analyses.

O-*Methylation of Fractions* 1, 3, 4, 5, 6, 7, *and* 10 *combined.*—By exhaustive methylation of these combined fractions (535 mg.) in a similar manner to the above, there was no separation of any iodide until the solution had been concentrated. A sparingly soluble iodide C (32 mg.), unmelted at 295°, then separated in anisotropic nodules. The main bulk of material was, however, readily soluble in methyl alcohol, but could be fractionally precipitated by addition of water with separation of a succession of gums which on re-solution in methyl alcohol slowly deposited clusters of straw-coloured needles. The various crystalline fractions of similar appearance were collected and crystallised twice from methyl alcohol, the pure *iodide B*, m. p. 318° (40 mg.), separating in clusters of cream-coloured needles (Found, in salt dried at 100° in a high vacuum: C, 51·7, 51·9; H, 5·5, 5·5; N, 3·4, 3·6; I, 32·1, 32·1; MeO, 13·8, 13·7. $C_{17}H_{22}O_2NI$ requires C, 51·1; H, 5·7; N, 3·5; I, 31·8; 2MeO, 15·5%. $C_{18}H_{22}O_2NI$ requires C, 52·5; H, 5·4; N, 3·4; I, 30·9; 2MeO 15·1%). This iodide is very similar in its properties to *O*-methylprotocuridine methiodide. It has the same m. p. and does not depress the m. p. of the latter. It is not very soluble in hot water and gives no precipitates with metaphosphoric acid or nitric acid. Although the duplicate microanalytical figures show agreement among themselves but diverge considerably from those of *O*-methylprotocuridine methiodide, the very close similarity of the two salts suggests identity, and it is possible that in the " quaternary fraction " there is a new soluble phenolic quaternary chloride which on *O*-methylation gives *O*-methylprotocuridine methiodide. The final aqueous liquor from the methylation containing water-soluble quaternary salts was freed from iodide by silver chloride and then freed from inorganic salts by drying and alcohol extraction. The residual solid (89 mg.) obtained on removal of alcohol had a frog-paralysing dose of 6·9 mg. per kg. in 17 minutes.

Boehm's Metaphosphoric Acid Reaction.—Boehm found that metaphosphoric acid solution gave a precipitate with the alkaloids from pot-curare and also with curine from tube-curare. This was interpreted as showing that pot-curare contains alkaloids of the curine group. Whilst this deduction is correct, as the present communication has demonstrated, the argument is not a sound one, since metaphosphoric acid solution gives precipitates with the salts of a large number of alkaloids, *e.g.*, coclaurine, bebeerine methochloride, ergotoxine, isoemetine, brucine, and demethoxyemetine, but not with protopine or papaverine. It is essential that the metaphosphoric acid solution should be prepared from freshly ignited acid, since phosphoric acid does not give these reactions.

The Millon Reaction.—A positive reaction with the Millon reagent is readily given by phenols with one other substituent, as *e.g.*, guaiacol and the three cresols. On the introduction of another substitutent the reaction becomes more specific, for it is not given by thymol, carvacrol, α-naphthol, or *iso*vanillic acid, all of which have free positions *o*- and *p*- to the phenolic group. The reaction is, however, given by β-naphthol, vanillin, and methyl vanillate, which have at least one free *o*-position, but in which the *p*-position is occupied. When there are three substituents besides the phenolic group, the reaction is not given in the case of corybulbine, which is an *iso*quinoline alkaloid of the corydaline group with an *iso*quinoline group substituted in 6 and 7 by phenolic and methoxy-groups respectively. A positive reaction is, however, given by the bisbenzyl*iso*quinoline alkaloids, bebeerine and tubocurarine chloride, and by protocuridine but not by neoprotocuridine. From these results it seems legitimate to conclude that in a bisbenzyl*iso*quinoline alkaloid of the types shown in (I), (II), and (III), no Millon reaction would be given by (I) or (II), since the reaction with corybulbine is negative, but if either (or both) of the central methoxy-groups in (II) was made phenolic, a positive reaction would be given, since vanillin and methyl vanillate give the reaction. For similar reasons (III) would give a positive Millon reaction only if the phenolic group was in the position shown. It follows that, in tubocurarine chloride and in bebeerine which have structures based on (III), one of the phenolic groups is in the central benzyl nucleus as shown. This argument, if valid, leads to a relative orientation of phenolic and methoxyl groups in tubocurarine different from that provisionally assigned in Part I of this series (*loc. cit.*, p. 1384).

Examination of Strychnos Castelnaei *Wedd.*—The powdered bark (79 g.), percolated with 1% tartaric acid (1400 c.c.), showed a weak reaction with Tanret's reagent. The solution was

purified with basic lead acetate solution (200 c.c.), and the filtrate freed from lead and concentrated (17 c.c.). On the frog this solution had a true curare activity corresponding to 0·8 g. of original bark per kg. of frog, paralysis occurring in 8 minutes.

The alkaloidal solution was made alkaline with sodium bicarbonate (5 g.) and thoroughly extracted with chloroform. The alkaloidal bases from the latter were converted into amorphous hydrochlorides (155 mg.). These contained no phenolic bases, and did not give a strychnine-like colour reaction. The bicarbonate mother-liquor was neutralised, made up to 100 c.c., and precipitated by addition of powdered mercuric chloride (7·5 g.). The cream-coloured mercurichloride of the quaternary bases was freed from mercury, and the resulting solution of quaternary alkaloidal chlorides evaporated to dryness and extracted with absolute ethyl alcohol. The extracted chlorides (150 mg.) gave a purple colour with sulphuric acid and dichromate or ammonium vanadate. A dose of 1·5 mg. per kg. of frog paralysed in 14 minutes. It was converted into the sparingly soluble amorphous iodide, which had a paralytic dose of 2 mg. per kg. *St. Castelnaei* bark therefore contains about 0·4% of alkaloidal bases, one-half of which are non-quaternary and one-half quaternary.

Examination of St. guianensis (*Aubl.*) *Baill.*—The bark of this species of *Strychnos*, No. 2467 in the British Guiana Forest Department Records, when percolated with 1% tartaric acid solution, gave a very dark solution unlike that obtained from any *Strychnos* hitherto examined. The extract gave a weak Tanret reaction only in the presence of mineral acid. The substance responsible for this reaction appeared to be a complex acid removable by basic lead acetate. The concentrated original tartaric acid extract when neutralised showed no curare action on a frog within 1 hour in a dose corresponding to 20 g. of bark per kg. of frog.

National Institute for Medical Research, Hampstead. [*Received, June 15th,* 1937.]