174. *Curare Alkaloids. Part VI. Alkaloids from* Chondrodendron tomentosum *R. and P.*

By Harold King.

d-Tubocurarine chloride is of value in anæsthesia. The discovery of its botanical origin to ensure future supplies is therefore of importance. *Chondrodendron tomentosum* Ruiz and Pavon has been examined and found to yield *l*-curine and l-*tubocurarine chloride*. Dutcher (*J. Amer. Chem. Soc.*, 1946, **68**, 419) has also examined tube-curare prepared on the Upper Amazon from *Ch. tomentosum* but found *d*-tubocurarine chloride, *l*-curine, and other non-quaternary bases. It is possible that undifferentiated species are covered by the name *Ch. tomentosum*.

In 1935 it was shown (King, *J.*, 1381) that the active principle of tube-curare of native origin was crystalline *d*-tubocurarine chloride. The botanical origin of the poison was not known, but the view was expressed that the genus *Chondrodendron* was probably involved. It was also known, from museum specimens and from the exploratory journeys of Poeppig in the early years of the last century, that tube-curare came from the upper waters of the Amazon in Peru. Through the kind co-operation of Mr. J. W. Massey of the British Consulate at Iquitos in Peru, I received in 1939 a quantity of the liane, " amphi huasca " (amphi = poison, huasca = rope), used by the Indians near Tarapoto in the preparation of curare, which was collected by the late Guillermo Klug. At the same time I also received ample specimens of the leaves of the same liane, and these were identified by Mr. N. Y. Sandwith, M.A., of The Herbarium, Kew, as being indistinguishable from the leaves of *Ch. tomentosum* R. and P. They agreed perfectly with leaves (herbarium sheet No. 4474) collected by Spruce at Tarapoto in 1856 and identified as *Ch. tomentosum* by Moldenke (Krukoff and Moldenke, *Brittonia*, 1938, **3**, 16).

The powdered stem was extracted with dilute tartaric acid; a sample of the neutralised extract had a true curare action when tested on the frog. The extract was worked up for non-quaternary and quaternary alkaloids in the usual way; the non-quaternary fraction readily yielded *l*-curine (*l*-bebeerine) identical with the alkaloid isolated from natural tube-curare (King, *loc. cit.*). The quaternary fraction crystallised with difficulty but was eventually obtained pure and proved to be, not *d*-tubocurarine chloride as expected, but l-*tubocurarine chloride*.

Folkers and Unna (*Arch. int. Pharmacodyn.*, 1939, **61**, 373) examined a small specimen of *Ch. tomentosum* collected by Klug and identified by Krukoff, and found evidence of definite curare-like action in the crude quaternary fraction. Wintersteiner and Dutcher (*Science*, 1943, **97**, 467; Dutcher, *J. Amer. Chem. Soc.*, 1946, **68**, 419), however, were able to examine a sample of curare, prepared by Indians of the Upper Amazon, in which only one plant species was used. This was said to have been identified as *Ch. tomentosum* by a botanist at the time of preparation and authenticated by herbarium specimens. From this curare they isolated four non-quaternary bases, one of which was identified as *l*-curine, and a quaternary crystalline alkaloid which proved to be *d*-tubocurarine chloride.

The conclusion to be drawn from these observations, provided the botanical identification of the raw materials has been correct, is that under the name *Ch. tomentosum* there must be two closely allied species which need further differentiation by systematic botanists, one of which yields *l*-curine and *l*-tubocurarine chloride and the other *l*-curine and *d*-tubocurarine chloride. The position is reminiscent of that which held for " pareira brava " until a few years ago (King, *J.*, 1940, 737). Sometimes this drug gave *d*-bebeerine and sometimes *l*-bebeerine, and this was traced to the fact that two closely allied and difficultly distinguishable *Chondrodendron* species were concerned, one of which, *Ch. microphyllum*, gave *d*-bebeerine and the other, *Ch. platyphyllum*, *l*-bebeerine. It is not without interest that *Ch. toxicoferum* (Wedd, Krukoff and Moldenke) (*Ch. polyanthum*, Diels) bears a superficial resemblance to *Ch. tomentosum* and grows like the latter in the Huallaga river valley of the Upper Amazon. Chemical examination of this species is much to be desired.

Experimental.

The stem of *Ch. tomentosum* (667 g.) was powdered and percolated with 1% tartaric acid (10 l.) and the solution then concentrated to 1 l. This solution when neutralised was tested for curare activity on the frog; the paralysing dose corresponded to 1·0 g. of stem per kg. of frog (compare King, *J.*, 1937, 1478, for definition).

A preliminary assay for alkaloidal content and distribution of activity was carried out on the main solution. Of this, 10 c.c. were treated with chloroform (50 c.c.) and saturated sodium hydrogen carbonate solution (15 c.c.). An amorphous precipitate which formed was removed by filtration, and the aqueous layer was extracted with 3 further portions of chloroform. The chloroform on evaporation

left a syrup which on being moistened with methyl alcohol crystallised, yield 0·5 g. This proved to be *l*-curine, m. p. 213°. The rotation of the dried material was determined in N/10-hydrochloric acid; $[\alpha]_{5461}$ − 313°. Pure *l*-curine under similar conditions gave $[\alpha]_{5461}$ − 340° (King, *J.*, 1940, 742). The rotation liquor on evaporation readily crystallised giving *l*-curine hydrochloride, m. p. 282° (efferv.) not depressed by admixture with pure *l*-curine hydrochloride.

The sodium hydrogen carbonate mother liquor and the amorphous precipitate, which had been collected, were separately neutralised with sulphuric acid and assayed by the frog test. It was found that there was approximately twice as much activity in the precipitate (phenolic betaine formation) as in the mother liquor.

On this basis, the main bulk of solution was worked up in a similar way. The total yield of crystalline *l*-curine was 34·1 g. corresponding to a 5% yield on the original stems.

The combined amorphous precipitates produced by bicarbonate and insoluble in chloroform were dissolved in N/10-sulphuric acid, concentrated to 1150 c.c., and treated with sulphuric acid (57 g.) followed by 25% phosphotungstic acid in 5% sulphuric acid. The precipitate was converted into the hydrochloride in the usual way, and the solution concentrated to 70 c.c. and treated with saturated aqueous mercuric chloride solution (70 c.c.) followed by addition of solid mercuric chloride (15 g.). The precipitate was decomposed with hydrogen sulphide and the filtered solution evaporated to dryness, yield 5·7 g. (*A*).

In a similar way the sodium hydrogen carbonate mother liquor was neutralised, concentrated to 800 c.c., precipitated with phosphotungstic acid solution, converted into chloride, and precipitated with mercuric chloride. The mercury-free water soluble residue amounted to 5·6 g. (*B*).

These two fractions of comparable weights, containing the quaternary alkaloids, were kept as aqueous syrups at 0° for some months; that from the amorphous phenolic-betaines (*A*) had then crystallised partially. The solid was collected, yield 0·74 g. It was crystallised twice from water and gave l-*tubocurarine chloride*, minute needles, m. p. 275° (efferv.) (Found on hydrated material: loss at 100° (macro), 10·7; (micro), 9·6. OMe (micro), 7·0. $C_{38}H_{44}O_6N_2Cl_2,5H_2O$ requires H_2O, 11·5; loss of $4H_2O$, 9·2; 2OMe, 7·9%. Found on dried material (micro): C, 63·5; N, 6·5. $C_{38}H_{44}O_6N_2Cl_2,H_2O$ requires C, 63·9; H, 6·5%). Partial loss of water of crystallisation on micro-drying had been observed previously for *d*-tubocurarine chloride (King, *J.*, 1935, 1386). The anhydrous salt showed $[\alpha]^{20°}_{5461}$ − 258° (*c*, 0·38). The rotation of *d*-tubocurarine chloride was determined afresh under comparable conditions and gave $[\alpha]^{20°}_{5461}$ + 256° (*c*, 0·39) for the anhydrous salt.

The mother liquors of the *l*-tubocurarine chloride were fractionally precipitated with saturated aqueous mercuric chloride and seven fractions collected. Each of these was freed from metallic ions, but no further crystallisation of *l*-tubocurarine chloride could be effected. Each of these fractions was then completely methylated in methyl alcoholic solution with methyl iodide in excess and sodium methoxide, and each gave complex amorphous quaternary iodides. From fraction 6 a crystalline solid was obtained which on further crystallisation from water gave l-O-*methyltubocurarine iodide* clusters of small prisms, m. p. 268° (efferv.), yield 22 mg. (Found: Loss at 95°, 5·5. $C_{40}H_{48}O_6N_2I_2,3H_2O$ requires H_2O, 5·6%. Found for the anhydrous salt: C, 51·7; H, 5·6; OMe, 13·8. $C_{40}H_{48}O_6N_2I_2$ requires C, 52·9; H, 5·3; 4OMe, 13·7%). The figure for carbon is low but the same figure was found in duplicate micro-analyses of the dextro-form (King, *J.*, 1935, 1386). *d-O*-Methyltubocurarine iodide had previously been found to melt at 266—267° (efferv.).

It is probable that the *l-O*-methyltubocurarine iodide has arisen from *l*-tubocurarine chloride present in the mother liquors.

The fraction, 5·6 g. (*B*) above, failed to give *l*-tubocurarine chloride by direct crystallisation. It has not been examined further chemically, but biological assay showed that it contains a more powerful curarising agent than *l*-tubocurarine chloride.

I am indebted to Dr. B. D. Burns of this Institute for a biological assay of *l*-tubocurarine chloride, which, on the isolated rat's diaphragm, was 30 to 60 times weaker than *d*-tubocurarine chloride. The quaternary fraction (*B*) was about 3·5 times weaker than *d*-tubocurarine chloride, but the active principle contained in it cannot be *d*-tubocurarine chloride.

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