394. *Curare Alkaloids. Part VIII. Examination of Commercial Curare,* Chondrodendron tomentosum *R and P and* Anomospermum grandifolium *Eichl.*

By Harold King.

The aim of the present investigation was primarily the determination of the botanical source of *dextro*tubocurarine chloride on account of the use of the latter as an adjunct in anæsthesia. A bush-rope from northern Peru has now given *dextro*tubocurarine chloride and four non-quaternary bases of which *dextro*tomentocurine is new. A commercial curare from southern Peru also prepared from *Ch. tomentosum* has likewise given *dextro*tubocurarine chloride and four non-quaternary alkaloids. These results are in conflict with the results obtained in Part VI where *Ch. tomentosum* from northern Peru gave *lævo*tubocurarine chloride. A botanically undifferentiated species may be involved.

Anomospermum grandifolium which has a reputation as a constituent of curare has been examined. It contained a quaternary fraction with a curare-action.

In Part I (King, *J.*, 1935, 1381) it was shown that the active principle of tube-curare was *dextro*tubocurarine chloride. Since that time this substance has found important application

as an adjunct in surgical anæsthesia, and to ensure supplies of this drug it was essential to identify the plant which produced this alkaloid. It was known that tube-curare came from the region of the upper waters of the Amazon in Peru and from the chemical relationship established between *dextro*tubocurarine chloride and bebeerine it was thought that the genus *Chondrodendron* was involved.

In Part VI (King, *J.*, 1947, 936) an account was given of the chemical examination of stems of *Chondrodendron tomentosum* collected near Tarapoto, in northern Peru. Leaves which were collected at the same time were found by Mr. N. Y. Sandwith, M.A., of The Herbarium, Kew to be indistinguishable from authentic leaves of this species. On chemical examination the stems yielded *lævo*bebeerine (*lævo*curine), a tertiary alkaloid found accompanying *dextro*-tubocurarine chloride in tube-curare, and *lævo*tubocurarine chloride.

Dutcher (*J. Amer. Chem. Soc.*, 1946, **68**, 419), however, from a sample of curare prepared by Indians of the upper Amazon under the supervision of a botanist-explorer who identified the plant used as *Chondrodendron tomentosum*, isolated four tertiary alkaloids, *dextroiso*chondrodendrine, *dextroiso*chondrodendrine dimethyl ether, *lævo*bebeerine, and *dextro*chondrocurine. The quaternary alkaloid proved to be *dextro*tubocurarine chloride.

Thus the plant *Chondrodendron tomentosum* has on one occasion given *lævo*tubocurarine chloride and on another the *dextro*-alkaloid. Similarly the *radix pareiræ bravæ* of pharmacy sometimes yielded *dextro*bebeerine and at other times *lævo*bebeerine. It was found (King, *J.*, 1940, 737) that when *pareira brava* gave *lævo*bebeerine it came from *Chondrodendron platyphyllum* and when it gave *dextro*bebeerine from *Ch. microphyllum*. It seems very probable therefore that two species are involved under the name *Ch. tomentosum* and it is only right to add that my adviser on botanical matters, Mr. Sandwith, has always emphasised that final and conclusive identification of a species cannot be made on sterile botanical specimens, that is on specimens without flowers. On the present evidence another and possibly new *Chondrodendron* species must be implicated but whether it yields *dextro*- or *lævo*-tubocurarine chloride must for the present remain undetermined.

Since the above results were obtained, further material has been received from Peru and its examination is the subject of this communication.

To meet the demand for *dextro*tubocurarine chloride, the firm of Asher, Kates y Cia, S.A., of Lima, Peru, collect the stems of a bush-rope which grows in the region of the Madre de Dios River, in the province of Cuzco, Peru, and make a concentrated extract. This firm kindly supplied me with leaves of the plant they use and these were identified by Mr. Sandwith as being indistinguishable from *Ch. tomentosum*. A sample of the crude concentrated extract furnished *dextro*tubocurarine chloride, isolated by use of ammonium reineckate by Kapfhammer's process so as to avoid the strongly acid solutions involved in the mercuric chloride process. The non-quaternary alkaloids were separated by a method depending on their different basicities (see King and Ware, *J.*, 1941, 331) into the four alkaloids, *iso*chondrodendrine dimethyl ether, *lævo*bebeerine, *dextro*chondrocurine, and *dextroiso*chondrodendrine, the basicity increasing in the order given. It is of interest that these five alkaloids are the same as those found by Dutcher (*loc. cit.*) who separated the non-quaternary ones by chromatography. It is possible that Dutcher's starting material came from the same region as this commercial extract as the term Upper Amazon used by Dutcher would cover the Madre de Dios River.

Through the kind co-operation of Mr. J. W. Massey, of the British Consulate at Iquitos in northern Peru, I received in April 1947, a quantity of stems and leaves, collected at Sisa in the region of Tarapoto, of a bush-rope said to be used by the natives in the preparation of an arrow-poison. It will be recalled that it was from Tarapoto that the stems and leaves, identified as *Ch. tomentosum*, and which gave *lævo*tubocurarine chloride, came (see Part VI). The new material was also identified by Mr. Sandwith as being indistinguishable from *Ch. tomentosum*. Chemical examination showed the presence of five alkaloids, *dextro*tubocurarine chloride, *lævo*-bebeerine, *dextro*chondrocurine, *dextroiso*chondrodendrine, and a new alkaloid dextro*tomentocurine*. The latter is the most strongly basic of the non-quaternary alkaloids. It only occurred in very small quantity and in its properties resembled *dextroiso*chondrodendrine more closely than it did *lævo*bebeerine and *dextro*chondrocurine. A special search was made, in the non-phenolic fraction, for *iso*chondrodendrine dimethyl ether but it was not found. Four of the alkaloids agree with those found in the commercial sample of curare from the province of Cuzco and with those found by Dutcher. Two of them, *dextro*tubocurarine chloride and *lævo*bebeerine (*lævo*-curine), also agree with the alkaloidal bases found in the original tube-curare of native origin described in Part I.

It may therefore be regarded as established that from widely separated Peruvian localities a bush-rope indistinguishable from *Ch. tomentosum* yields *dextro*tubocurarine chloride. Another

bush-rope also indistinguishable as far as its leaves are concerned from *Ch. tomentosum* has yielded *lævo*tubocurarine chloride, an enantiomorph of inferior activity. It is of interest that Foster and Turner (*Quart. J. Pharm. Pharmacol.*, 1947, **20**, 228), examining commercial samples of curare purporting to yield the *dextro*-alkaloid, have on one occasion also encountered a *lævo*rotatory quaternary salt of inferior activity.

It is unlikely that further progress in unravelling this problem can be made until flowers and stems of the two species concerned can be obtained and their identity established beyond doubt.

The botanist Schwacke (*Jahrb. Kgl. bot. Gart. bot. Mus., Berlin*, 1881—4, III, 220) identified one of the ingredients of Ticuna curare as *Anomospermum grandifolium* Eichl [now known as *Elissarrhena grandifolia* (Eichl) Diels]. De Lacerda (*Arch. Mus. nac.*, Rio de Janeiro, 1901, **11**, 163) examined this species and found that a crude extract had a true curare action. Krukoff and Moldenke (*Brittonia*, 1938, **3**, 73), however, expressed their conviction that the plant identified as *Anomospermum grandifolium* by Schwacke was in reality *Chondrodendron limaciifolium*. Mr. J. W. Massey kindly sent me a quantity of leaves and stems of a liane, known in the Iquitos region as amphi-huasca (poison-rope). This was identified by Mr. N. Y. Sandwith as being indistinguishable from *Anomospermum grandifolium* Eichl. A crude extract of the stems was found by Dr. F. C. MacIntosh of this Institute to have a true curare action and when the alkaloids were separated into quaternary and non-quaternary bases the curare activity was found in the quaternary fraction. There is thus this further experimental evidence in support of the occasional use of *Anomospermum grandifolium* by the natives as an active ingredient of curare.

Experimental.

Commercial Curare from Lima, Peru.—Through the kindness of Mr. Herbert A. Berens of Biddle Sawyer & Co., a sealed sample tin of curare was obtained from Asher Kates & Co., of Lima, who supply the British and American markets with crude curare from which *dextro*tubocurarine chloride is prepared. The product, a thick black syrup weighing 84 g., was made up to 1165 c.c. with water and when assayed by Dr. F. C. MacIntosh of this Institute by the rabbit head-drop test exhibited curare activity equivalent to a 1·5% solution of *dextro*tubocurarine chloride.

Isolation of dextro*Tubocurarine Chloride.*—The solution was treated with basic lead acetate solution (250 c.c., 0·5N), the precipitate removed, and the filtrate freed from lead by hydrogen sulphide. An aliquot portion (500 c.c.) of the filtrate (2060 c.c.) was treated with sodium hydrogen carbonate (21 g.) and kept for 12 hours and then filtered from the precipitate (4·4 g.). The filtrate was extracted 6 times with chloroform which removed non-quaternary bases (1·5 g.). The extracted solution was neutralised with hydrochloric acid (30 c.c.; *d* 1·12) and treated with a saturated solution of ammonium reineckate (300 c.c.). The precipitated reineckate was collected and whilst still damp dissolved in acetone and the acetone solution treated with excess of saturated silver sulphate solution. The silver reineckate was removed and the filtrate treated carefully with barium chloride solution to remove all sulphate and silver ions. The combined solutions, from the 4 aliquot portions, on concentration, gave crude *dextro*tubocurarine chloride (6·4 g.). A portion (1·73 g.) recystallised from water gave *dextro*tubocurarine chloride (1·59 g.), $[\alpha]_D^{17°}$ +199°. Dutcher (*J. Amer. Chem. Soc.*, 1946, **68**, 421) gives $[\alpha]_D^{22°}$ +215° for the pure anhydrous salt.

*Isolation of Dimethyl*iso*chondrodendrine,* lævo*Bebeerine,* dextro*Chondrocurine, and* iso*Chondrodendrine.*—The precipitates obtained with sodium hydrogen carbonate from the 4 aliquot portions were mixed and ground with chloroform to remove non-quaternary bases. The chloroform-soluble portion was mixed with the main chloroform extracts containing the non-quaternary bases and, on removal of solvent, gave 12·8 g. of non-quaternary bases which on titration required 30·5 c.c. of N-hydrochloric acid for neutralisation. This solution was extracted with chloroform to remove fat (0·3 g.) and then the alkaloids were liberated fractionally in the presence of chloroform by addition of 14 successive portions (each 2·5 c.c.) of N-sodium carbonate solution. Each of the 14 chloroform extracts was evaporated to dryness and moistened with methyl alcohol. Fractions 5 to 9 readily crystallised, the solid in each case proving to be *lævo*bebeerine, m. p. 212° not depressed by admixture with authentic *lævo*bebeerine and raised to 287° by admixture with *dextro*bebeerine, total yield 2·24 g. Fraction 8 on keeping deposited *dextro*chondrocurine, m. p. 236°, and fraction 9 later deposited *iso*chondrodendrine (0·12 g., m. p. 309°) and still later *dextro*chondrocurine (0·25 g., m. p. 240°). Fractions 10 to 12 gave *iso*chondrodendrine (1·02 g., m. p. 305°).

The methyl alcoholic mother liquors of fractions 3 to 9 were then mixed, the solvent removed, and the alkaloidal residue titrated with N-hydrochloric acid (7·5 c.c.) and again fractionally liberated into chloroform by the successive addition of 13 portions, each of 0·75 c.c. of N-sodium carbonate solution. On removal of the chloroform from each, the residue was moistened with methyl alcohol as before and all fractions crystallised with the exception of the first. Fractions 3 to 5 gave *lævo*bebeerine (0·3 g.), fractions 6 to 8 gave crude *dextro*chondrocurine (0·37 g.), whilst fractions 10 to 13 gave *iso*chondrodendrine (90 mg.). Fraction 2 contained a readily-soluble alkaloid crystallising in clear tablets, m. p. 274°. It gave no Millon reaction, was non-phenolic, and was stable to warm dilute nitric acid. These properties agree very well with those recorded by Dutcher (*loc. cit.*) and Kondo, Tomita, and Uyeo (*Ber.*, 1937, **70**, 1890) for *dextroiso*chondrodendrine dimethyl ether.

The combined crops of *lævo*bebeerine (2·55 g.) were converted into the hydrochloride (2·42 g., m. p. 275° efferv.). In a similar way the combined crops of *iso*chondrodendrine (1·23 g.) were neutralised with sulphuric acid and gave the very characteristic octahedra of *iso*chondrodendrine sulphate (1·21 g., m. p. 290° efferv.). The combined fractions of *dextro*chondrocurine (0·64 g.) were converted into the *hydrochloride*, m. p. 288° (efferv.), yield 0·41 g. This salt crystallised in elongated hexagonal plates which at first contained 6·5 molecules of water of crystallisation, but after a few days this had fallen to 2·5

molecules (Found, on fresh air-dried salt: loss at 110°, 14·5. $C_{36}H_{38}O_6N_2,2HCl,6{\cdot}5H_2O$ requires H_2O 14·9%. On further air-drying: loss at 100°, 6·5. $C_{36}H_{38}O_6N_2,2HCl,2{\cdot}5H_2O$ requires H_2O, 6·3%. On solid dried at 100°: C, 64·3; H, 6·3; MeO, 8·9. $C_{36}H_{38}O_6N_2,2HCl$ requires C, 64·7; H, 6·0; 2MeO, 9·3%). The rotation of the salt was determined in water, $[\alpha]_D^{20°}$ +208·7°, for the anhydrous salt (*c*, 0·57), whence $[\alpha]_D^{20°}$ +233° for the ion. Dutcher (*loc. cit.*) gives $[\alpha]_D^{24°}$ +193° for anhydrous *dextro*chondrocurine sulphate whence $[\alpha]_D^{24°}$ +224° for the ion. The base was recovered from the hydrochloride and crystallised from methyl alcohol, yield 0·24 g., m. p. 236—238° (Found: MeO, 10·4, 10·8. Calc. for $C_{36}H_{38}O_6N_2$: 2MeO, 10·4%). Dutcher (*loc. cit.*) gives m. p. of the base 232—234°.

The above mentioned sodium hydrogen carbonate precipitate (9·5 g.) which had been extracted with chloroform to remove all soluble bases was treated with N-hydrochloric acid (60 c.c.) and a tarry fraction removed. It was precipitated with ammonium reineckate and the precipitate converted into the chloride. Ammonia was added to remove chromium as hydroxide and the solution on concentration eventually deposited a further quantity (0·49 g.) of of *dextro*tubocurarine chloride.

Chondrodendron tomentosum *Stems from Tarapoto, Peru.—Chondrodendron tomentosum* stems (1·08 kg.) were received from Mr. H. W. Massey. They were collected at Sisa near Tarapoto and leaves were obtained at the same time and were identified by Mr. N. Y. Sandwith, M.A., as being indistinguishable from *Ch. tomentosum* leaves.

The stems were powdered, extracted with 15 litres of 1% tartaric acid, and the extract concentrated to 3·6 litres. Of this solution a portion (250 c.c.) was treated with basic lead acetate solution (100 c.c., 0·5N) and the filtrate freed from lead by passing hydrogen sulphide. The filtrate (500 c.c.) freed from lead sulphide and excess of hydrogen sulphide required 25 g. of sodium hydrogen carbonate to make the solution alkaline. After standing for a few days the solid A (1·93 g.) was removed and the aqueous solution extracted repeatedly with chloroform which removed 0·18 g. (B) of non-quaternary alkaloid. The extracted aqueous solution was made weakly acid with concentrated hydrochloric acid (27·5 c.c.) and treated with saturated ammonium reineckate solution (100 c.c.). The precipitated reineckate was converted into chloride and the solution on concentration gave 0·37 g. of crystalline *dextro*tubocurarine chloride; $[\alpha]_D^{20°}$ +202° (*c*, 0·53), for the anhydrous salt. A small second crop (0·1 g.) was obtained from the mother liquor.

The solid A (1·93 g.) was treated with chloroform which removed 0·92 g. of non-quaternary bases which were mixed with fraction B (0·18 g.) and dissolved in methyl alcohol. On keeping, crystalline microscopic spheroids of *dextroiso*chondrodendrine separated (80 mg.), m. p. 306°. It gave the very characteristic sulphate crystallising in glassy octahedra, and the dextro-rotation was confirmed by neutralising the base (80 mg.) with N/10-sulphuric acid, when $[\alpha]_D^{17°}$ was +140·6° calculated in terms of the base.

The chloroform-insoluble solid (1·01 g.) was neutralised with N-hydrochloric acid (5·0 c.c.), treated with saturated ammonium reineckate solution (40 c.c.) and the solid collected and converted into chloride. The solution on concentration deposited *dextro*tubocurarine chloride (0·32 g.), $[\alpha]_D^{21°}$ +195·5° (*c*, 0·4), for the anhydrous salt.

Isolation of lævo*Bebeerine*, dextro*Chondrocurine*, dextroiso*Chondrodendrine, and Tomentocurine from the Non-quaternary Bases.*—The remainder (3350 c.c.) of the original tartaric acid extract was cleaned up with basic lead acetate and concentrated to 2000 c.c. This was made alkaline by addition of sodium hydrogen carbonate and the precipitated solid A removed after keeping for 24 hours. The filtrate was repeatedly extracted with chloroform which removed the remainder (2·0 g.) of the non-quaternary bases. The precipitated solid A, was dried in a vacuum and extracted with chloroform which removed 14·0 g. of non-quaternary bases. The combined crops of non-quaternary bases were neutralised with N-hydrochloric acid (42 c.c.) and then divided into 16 chloroform-soluble fractions by first extracting with chloroform to remove fat and then with chloroform after addition of 15 portions, each of 3 c.c., of N-sodium carbonate solution. The average weight of base in each chloroform-soluble fraction was about a gram. The 15 basic fractions were each dissolved in methyl alcohol (3—4 c.c.) and kept. Fractions 3 to 5 deposited *lævo*bebeerine (1·45 g.), m. p. 212°, undepressed by an authentic sample, and giving the hydrochloride (0·97 g.), m. p. 278° (efferv.), $[\alpha]_{5461}^{20°}$ −259° (*c*, 0·52) for the anhydrous salt. Fractions 8 to 13 gave *dextroiso*chondrodendrine (1·66 g.), m. p. 306—310°. It was converted into the sulphate (1·69 g.), m. p. 292° (decomp.), and gave $[\alpha]_D^{20°}$ +113·6°; $[\alpha]_{5461}^{20°}$ +145·7° for the anhydrous salt. On more prolonged keeping, fractions 6 to 10 all deposited *dextro*chondrocurine (1·45 g. in all), m. p. 237—238°, which was converted into the hydrochloride (1·35 g.), m. p. 285—286°. It lost 7·7% on drying, corresponding to 3 molecules of water of crystallisation, and had $[\alpha]_{5461}^{20°}$ +247°; $[\alpha]_D^{20°}$ +210° for the anhydrous salt (*c*, 0·46). This agrees with the value $[\alpha]_D^{20°}$ +208·7° found for *dextro*chondrocurine hydrochloride isolated from commercial curare (described above).

On more protracted keeping fractions 6 to 8 gave *lævo*bebeerine (0·94 g.). A special search was made for *iso*chondrodendrine dimethyl ether by combining the mother liquors of fractions 1 to 7 by evaporating to dryness and solution of the residue in chloroform. Phenolic alkaloids were removed by N-sodium hydroxide and on liberation to chloroform by passage of carbon dioxide gave a phenolic fraction of 3·2 g. The non-phenolic fraction left in the chloroform after alkaline extraction amounted to 0·29 g. but all attempts to crystallise *iso*chondrodendrine dimethyl ether from it failed. The phenolic fraction was again separated into 12 fractions of different basicity and gave *lævo*bebeerine (0·17 g.), *dextro*chondrocurine (0·59 g.), and *dextroiso*chondrodendrine (0·14 g.). Fractions 11 and 12, representing the most basic fractions, gave 75 mg. of a new alkaloid, m. p. 260—270°.

As this new base appeared at the end of the *iso*chondrodendrine fractions it was of interest to examine the mother liquors of fractions 8 to 13 of the original scheme of fractionation. The combined alkaloidal bases from the evaporated methyl alcoholic solutions were neutralised with acid and fractionally liberated to chloroform in 10 fractions from which *dextro*chondrocurine (0·18 g.) and *dextroiso*chondrodendrine (0·11 g.) were obtained and finally from the most basic fraction 10, a further 60 mg. of the new alkaloidal base, m. p. 265°.

The three fractions (135 mg. in all) of the new alkaloidal base for which the name *tomentocurine* is proposed were combined and neutralised with N/10-sulphuric acid (2·7 c.c.) and the solution evaporated. The characteristic octahedra of *dextroiso*chondrodendrine did not separate. The base was regenerated

to chloroform, the solvent evaporated to a small volume, and then methyl alcohol (5 c.c.) added. There was instantaneous crystallisation of tomentocurine base as a microcrystalline powder (70 mg.) similar in appearance to *iso*chondrodendrine but with m. p. 265° (efferv.). It gave a Millon reaction and was readily oxidised by dilute nitric acid thus resembling all the phenolic alkaloids of this group. On drying at 100° it lost 7% and the dried solid in N/10-hydrochloric acid had $[\alpha]_D^{17°}$ +210°; $[\alpha]_{5461}^{17°}$ +278·5° in terms of the base (*c*, 0·15). These rotations are far higher than those of *dextroiso*chondrodendrine. The hydrochloric acid solution was evaporated to a syrup but the hydrochloride could not be induced to crystallise.

The composition of the base (Found: C, 69·6; H, 8·2; N, 3·5, 3·9; OMe, 21·3%) was difficult to reconcile with a member of this group of alkaloids. Owing to the very small amounts of material available confirmation of the analytical figures has not been possible and they are not regarded as final.

From the main liquor containing quaternary bases, *dextro*tubocurarine chloride (3·8 g.) was isolated through the reineckate. The sodium hydrogen carbonate precipitate (A) from which chloroform-soluble alkaloids had been removed was neutralised with N-hydrochloric acid (85 c.c.) and treated with saturated ammonium reineckate solution (650 c.c.). The precipitated reineckate was converted into chloride and material precipitable by dilute ammonia removed in dilute solution. On concentration and inoculation the solution slowly deposited *dextro*tubocurarine chloride (1·6 g.) but not with the ease of the preliminary run on 250 c.c. of original stem extract.

The Rotation of dextroiso*Chondrodendrine.*—For two commercial samples of *iso*chondrodendrine sulphate Faltis and Neumann (*Monatsh.*, 1921, **42**, 321) give $[\alpha]_D^{21°}$ +135° and $[\alpha]_D^{16°}$ +114·7° for the anhydrous salt. In Part V (King, *J.*, 1940, 745) a value $[\alpha]_{5461}$ +115·6° was recorded for the anhydrous sulphate which corresponds to a value $[\alpha]_D$ +99·7° if Biot's law holds. Dutcher (*J. Amer. Chem. Soc.*, 1946, **68**, 423) gives a value $[\alpha]_D^{22°}$ +101° for the anhydrous salt. Earlier in this communication $[\alpha]_D^{20°}$ +113·6° and $[\alpha]_{5461}^{20°}$ +145·7° were recorded for anhydrous *iso*chondrodendrine sulphate which had been prepared from specimens of *iso*chondrodendrine obtained by a fractionation depending on different basicities. As this value was considerably higher than the figure given in Part V and the figure given by Dutcher, a sample of *dextroiso*chondrodendrine sulphate was recrystallised several times from water and gave $[\alpha]_{5461}^{19°}$ +158·9° (*c*, 0·7) for the anhydrous salt, whence $[\alpha]_D^{19°}$ +137° if Biot's law holds. This is a value very close to that given for a sample of the commercial sulphate by Faltis and Neumann.

Examination of Anomospermum grandifolium, *Eichl.*—The stems (484 g.) were extracted with 1% tartaric acid until the alkaloids were removed and the solution concentrated to 400 c.c. When tested by Dr. F. C. MacIntosh it was found, by the rabbit head-drop test, to have a curare action corresponding to that of a 0·01% solution of *dextro*tubocurarine chloride.

The alkaloids were then separated into chloroform-soluble non-quaternary bases and quaternary bases (precipitated by ammonium reineckate). The total chloroform-soluble bases amounted to 0·2 g. with a weak curare action when made up to 20 c.c. in neutral solution equivalent to a 0·006% solution of *dextro*tubocurarine chloride.

The quaternary fraction as chloride was made up to 10 c.c. and had an effect on the rabbit indistinguishable from that of a 0·2% solution of *dextro*tubocurarine chloride.

NATIONAL INSTITUTE FOR MEDICAL RESEARCH,
HAMPSTEAD, N.W. 3.

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