

Bisbenzyltetrahydroisoquinoline Alkaloids

III. Counter Current Separation and Crystallization of Methyl Ethers of *l*-Curine

ESKIL HULTIN*

Department of Pharmacology, University of California Medical Center, Los Angeles 24, California, U.S.A.

Dedicated to Professor *Holger Erdtman* on his 60th birthday

Partly methylated *l*-curine ("bebeerine", chondrodendrine) can easily be separated into one unmethylated and one methylated fraction with a counter current procedure using four separatory funnels and three volumes of ether and one volume of buffer pH 12.3. Moreover, the dimethyl ether of *l*-curine can also be easily separated from the fraction of monomethyl ethers at pH about 13.6, using four volumes of buffer and one volume of ether. The *l*-curine dimethyl ether, which has resisted all previous efforts to crystallize it, crystallized from diethyl ether, m.p. 172°C. One of the monomethyl ethers crystallized from benzene, m.p. 142°C, and could also be crystallized from diethyl ether in two forms, m.p. 132°C and 137°C, but did not crystallize from methanol. The other *l*-curine monomethyl ether crystallized from methanol, m.p. 148°C, but did not crystallize from benzene. The *l*-curine monomethyl ether m.p. 206–208°C previously obtained in small yield by Späth and Kuffner was not found, but a monomethyl ether fraction remained after the separation of the monomethyl ethers which had crystallized. The possibility of the natural occurrence of an isomer of *l*-curine is suggested.

The isolation of the alkaloid now called curine was first reported by Wiggers¹. From roots of a plant referred to as *Cissampelos pareira* he obtained an alkaloid which he called cissampelosine. Later he² isolated this substance from *radix pareirae bravae* which at that time was believed to originate from *Cissampelos pareira* L. He shortened the name of the substance to pelosine. A few years later Bödeker³ also investigated this substance as well as another alkaloid, bebeerine, which had recently been isolated from the bebeeru tree, *Nectandra rodioei* Hook., now called *Ocotea rodiei* (Hook.) Mez. Bödeker concluded that pelosine and bebeerine were different substances.

* Present address: Institutionen för organisk kemi, Tekniska högskolan, Stockholm 70, Sweden.

Some time later Hanbury^{4,5,57} came to the conclusion that *radix pareirae bravae* did not originate from *Cissampelos pareira* L. but from *Botryopsis platyphylla* (this name, however, was never formally proposed for the plant⁶), which is now called *Chondrodendron platyphyllum* (A. St. Hil.) Miers. Since Flückiger^{7, 8}, had been informed early about Hanbury's opinion, he made up and investigated an alkaloid preparation from *Chondrodendron platyphyllum* and also from a safely identified specimen of *Cissampelos pareira*, which he had obtained from Hanbury, and found pelosine in both plants. His results so far have been confirmed: pelosine, now called curine, was identified in *Cissampelos pareira* L. a few years ago⁹. Flückiger did not find any difference between pelosine and the bebeerine from *Nectandra rodioei* Hook., and he agreed with and earlier assumption that bebeerine also was identical with the so called buxine from *Buxus sempervirens* L. Later, it has been denied^{10, 11} and disproved¹²⁻¹⁵ that pelosine, bebeerine, and buxine are identical. However, the mistake was introduced into Hager's¹⁶ handbook*, where the preferred name is bebeerine; this substance had been sold in England for a long time as a remedy¹⁷. This seems to have influenced the commercial naming of certain alkaloid preparations of various botanical origins and thus differing chemical compositions; cf. Refs.¹⁸⁻²². The mistake about the identity also appears in *Die Pflanzenstoffe* by Husemann *et al.*²³, but there the oldest of the names for these alkaloids, buxine, is preferred, as suggested by Flückiger⁷.

Scholtz^{24,25} investigated a commercial, crude alkaloid preparation called *bebeerinum purum*, said to originate from *Nectandra rodioei*. He isolated an alkaloid — now called *l-curine* — which he found crystallized so remarkably easily from methanol that he even suggested²⁰ it be used as a reagent for methanol. However, Dutcher²⁶ and Anet *et al.*²⁷ had difficulties in crystallizing this alkaloid from methanol. Scholtz²⁸ obtained the same alkaloid from *radix pareirae bravae* and, finding support in Bödeker's investigation³ (which he quoted misleadingly as if Bödeker had discovered the identity of pelosine and bebeerine instead of finding a difference) and in Flückiger's investigation^{7, 8}, he considered the identity of bebeerine and pelosine proved. Subsequently Scholtz used the name bebeerine and not pelosine for the alkaloid from *radix pareirae bravae*. It now seems justifiable to doubt that the *bebeerinum purum* he investigated really originated from *Nectandra rodioei* (cf. Ref.¹⁷), particularly since he mentioned not *Nectandra rodioei* but *radix pareirae bravae* as its source in later papers^{20,22,29,30}, and in quoting from his original investigation²⁵ of *bebeerinum purum*; in some papers^{20, 30} he even stated that *Chondrodendron tomentosum* Ruiz & Pavon was the source of the root. This statement about which of the *Chondrodendron* species is the source of the root is supposed to be the consequence of a misunderstanding^{31,58}. A survey of the species of *Chondrodendron* and their geographical distribution has been given by Krukoff and Moldenke⁶ and by de Mello Filho³².

* "Bebeerinum, Bebeerin, Bebirin, Pelosin, Beberia (der Engländer), Buxin (C³⁸H⁴¹NO⁶ oder C₁₀H₂₁NO₃ = 311) ein Alkaloid, welches in der Rinde von *Buxus sempervirens* Linn., in der als Färbematerial benutzten Bibiririnde, Bebeerurinde, Rinde von *Nectandra Rodiaei* Schomburgk, einem in Guyana heimischen Baume aus der Familie der Laurineen, in der Pareirawurzel aufgefunden und als ein Ersatz des Chinins empfohlen wurde". For the peculiar first chemical formula here old atomic weights were used.

Shortly before Scholtz' first papers appeared about the alkaloid he called *d*-bebeerine, Böhm³³⁻³⁵ published his first results from an investigation of a South American arrow poison, called tubo curare because it was available in tubes of bamboo. Its botanical origin was unknown. Böhm found a substance to which he gave the name *l*-curine. He crystallized it from benzene (m.p. 161°C) and from methanol (m.p. 212°C). Although Böhm³⁵ and Scholtz²⁵ submitted each a paper to the same journal within an interval of only slightly more than 8 months, it was 30 years later before it was found — by Späth, Leithe and Ladek³⁶ — that Böhm's curine and Scholtz' "bebeerine" were *l*- and *d*-forms, respectively, of the same substance.

Scholtz obtained the *l*-form of curine in the beginning of his investigations, but later^{29,37} he obtained the *d*-form. It appears from King's³¹ work that this has to do with the origin of radix pareirae bravae: *Chondrodendron platyphyllum* (A. St. Hil.) Miers earlier, and *C. microphyllum* (Eichl.) Moldenke later. A third species, *C. tomentosum* Ruiz & Pavon is used by Indians of the upper Amazon for preparing curare^{26,58}

In 1933 Späth and Kuffner³⁸ tried to prepare the two monomethyl ethers of *l*-curine as part of a proof that its composition is $C_{36}H_{38}O_6N_2$ instead of $C_{18}H_{19}O_3N$ as previously assumed^{18,36}. Their efforts were only partly successful. The following procedure was used. Curine was partly methylated with diazomethane. The reaction product was dissolved in a mixture of ether and water containing potassium hydroxide; curine and its monomethyl ethers were extracted from the ether phase with a strong water solution of potassium hydroxide, whereas the dimethyl ether of *l*-curine remained in the ether phase. The separation of *l*-curine and its two monomethyl ethers was not described in detail, but it was mentioned that a tedious series of fractionated crystallizations from methanol and from diethyl ether was used, and that a procedure starting with a series of fractionated extractions was also used. Two different fractions of monophenol bases were finally obtained. One of them, in poor yield, was crystalline; m.p. 206—208°C. This substance was not found in the present investigation. The other was also crystalline, m.p. 154—160°C, but it was considered to still be a mixture of the *l*-curine monomethyl ethers, possibly containing *l*-curine.

The purpose of the present investigation was to elucidate the conditions for preparing the two *l*-curine monomethyl ethers from partly methylated *l*-curine. A similar work, in which the conditions for preparing the two *d*-chondrocurine monomethyl ethers were studied, has recently been reported by the present author³⁹. In each of these investigations the problem was divided into two main parts. The first part concerns separating the partly methylated alkaloid into three fractions according to the degree of methylation. The second part of the problem concerns separating the group of monomethyl ethers into its components.

THE USE OF ETHER AND BUFFER FOR THE SEPARATION OF PARTLY METHYLATED *l*-CURINE INTO GROUPS OF ISOMERS

Diethyl ether and buffered water give immiscible solvent systems suitable for the separation of partly O-methylated *l*-curine into three fractions: *l*-curine, the group of monomethyl ethers of *l*-curine, and the dimethyl ether of *l*-curine.

In these experiments, the buffer was made up of potassium phosphate, potassium sulfite (to inhibit as much as possible the formation of coloured substances) and potassium hydroxide. Sodium salts were avoided because a glass electrode was used in the measurements of pH. The partition coefficients for *l*-curine and its monomethyl ethers were measured at various pH values in those ranges which are of main interest in the separation procedure. The technique used was described in a previous paper⁴⁰ in this series. The results are given in Fig. 1, where the logarithms of the partition coefficients are plotted *versus* pH. For strongly alkaline buffer solutions the points for *l*-curine are close to a straight line with the inclination -2 , and the points for the monomethyl ethers of *l*-curine are close to straight lines with the inclination -1 , as can be expected from the presence of 2 and 1 phenolic hydroxyl groups, respectively, in these substances. The straight lines just mentioned are, of course, asymptotes.

It may be added here that the early problem^{18, 36, 38} about the molecular weight of curine, *i. e.*, whether $C_{18}H_{21}O_3N$ or $C_{36}H_{41}O_6N_2$ is the correct formula, could easily have been solved if the number of nitrogen atoms or phenolic hydroxyl groups which can participate in acid-base reactions had been computed from measurements of the partition coefficients between ether and a series of acid or alkaline buffers.

It was shown by Baker⁴¹ that a counter current separation of two substances is most efficacious when the ratio of the volumes of the two immiscible solvents equals the inverse value of the geometric mean between the partition coefficients of the two substances. In Fig. 1, where logarithms of partition coefficients for *l*-curine and its monomethyl ethers are given as curves, a line drawn midway between two such curves represents the negative value of the logarithm for the best ratio between the volumes of the two solvent phases. Thus, for a separation of *l*-curine and that monomethyl ether of *l*-curine, whose partition coefficient is nearer to that of *l*-curine, we can read from Fig. 1 that the pH value at which equal volumes of buffer and ether give the best separation

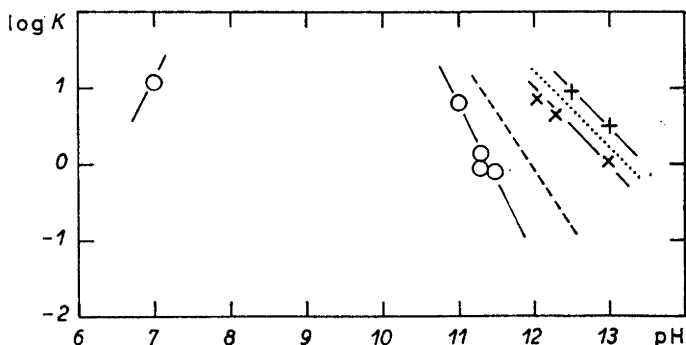


Fig. 1. The influence of pH on the partition coefficient K for *l*-curine (O) and its monomethyl ethers (\times and $+$) between diethyl ether and buffer. The dashed line gives the optimum relationship between volume ratio and pH for separation of *l*-curine from the one of its monomethyl ethers, whose partition coefficient is closest to that of *l*-curine, and the dotted line gives the optimum relationship between volume ratio and pH for separation of the two monomethyl ethers of *l*-curine.

tion (abscissa value zero) is about 11.95, and that, *e. g.*, at pH 12.3 the ordinate value is -0.5 and hence the logarithm for the optimum quotient obtained by dividing the volume of the less polar (ether) phase by the volume of the more polar (buffer) phase is $+0.5$, *i. e.*, 3 volumes of ether for 1 volume of buffer. We can also see from Fig. 1 that if we start the separation at pH about 12.3 with 3 volumes of ether and 1 volume of buffer, the difference in abscissa value for the line from which we read the best separation conditions and the line giving the value of the logarithm of the partition coefficient for *l*-curine is about 1.2 in the logarithmic scale. Hence, at pH 12.3 *l*-curine distributes itself in such a way that there will be about 16 times as much *l*-curine in the water phase as in the ether phase. Similarly, one of the monomethyl ethers distributes itself in such a way that there will be about 16 times as much of this substance present in the ether phase as in the water phase. A still larger fraction of the other monomethyl ether and of the dimethyl ether of *l*-curine will be present in the ether phase. The distribution coefficient of *l*-curine dimethyl ether was not measured, but it seems possible to assume safely from Fig. 1 that it is not below 1.5.

Distribution between the two solvent phases in one funnel thus gives a fair separation. The result can easily be greatly improved by adding a few, say two or three more separatory funnels for a counter current separation as previously described^{40, 42}.

Suitable conditions for an efficacious separation of *l*-curine dimethyl ether from the monomethyl ethers can also be read from Fig. 1. As previously mentioned, the value of the partition coefficient for the dimethyl ether of *l*-curine is probably not less than 1.5; it is more likely larger than 2. Results from several separations have not been contradictory to this. The buffer solution should have as high a pH value as conveniently possible, because the partition coefficient for that *l*-curine monomethyl ether which has the higher partition coefficient decreases with increased pH and is as large as 1 at pH 13.5. It is not too inconvenient to use a solution with pH about 13.6. If the volume of the buffer phase is 4 times as large as that of the ether phase, the monomethyl ether with higher partition ratio distributes itself in such a way that 5 times as much will be present in the water phase as in the ether phase. The other monomethyl ether distributes itself in such a way that about 15 times as much will be present in the water phase as in the ether phase, and the *l*-curine dimethyl ether can reasonably be expected to distribute itself in such a way that at least 10 times more will be present in the ether phase than in the water phase. This implies that in a so called "diamond separation" or "completion of squares" separation⁴² with four separatory funnels the recovery of the *l*-curine dimethyl ether in the ether phase can theoretically be expected to be better than 99.8%. In the water phase the recovery of the monomethyl ether with lower partition ratio can be expected to be still better, whereas the recovery of the monomethyl ether with higher partition ratio can be expected to be about 98%.

The various fractions are conveniently recovered as described in a previous paper³⁹: the pH value of buffer solutions is adjusted to about 8.5, and the precipitate thereby formed extracted with ether, whereby impurities remain undissolved. Ether solutions are extracted with dilute hydrochloric acid. The substance is precipitated from this water solution by adjusting the pH to

about 8.5, the precipitate is filtered off, washed with distilled water, the pH of which is adjusted to about 8.5, and dried *in vacuo*. It is possible to purify the preparation so obtained still more by extracting it with diethyl ether in a Soxhlet apparatus and evaporating the ether^{2, 25}.

THE USE OF ETHER AND BUFFER FOR COUNTER CURRENT SEPARATION OF *l*-CURINE MONOMETHYL ETHERS

In Fig. 1 two lines are drawn which show how the partition coefficients of the two *l*-curine monomethyl ethers between diethyl ether and buffer are influenced by pH. Halfway between these lines a dotted line is drawn which indicates how the optimum ratio between the two phases during a separation depends on pH. As previously discussed³⁹ in the similar problem of separating the two *d*-chondrocurine monomethyl ethers, a fair counter current separation is obviously possible even with a small number of transfers, but a difficulty is that the pH value of the buffer phase must be kept very close to its optimum value. Experiments on the separation of the monomethyl ethers of *l*-curine by a counter current method were discontinued when a convenient way to separate these substances by crystallization was found.

THE USE OF A FLEXIBLE FOUR COMPONENT SYSTEM

The partition coefficients for *l*-curine and its methyl ethers were also determined for various compositions of the four component solvent system previously³⁹ used in the chondrocurine series.

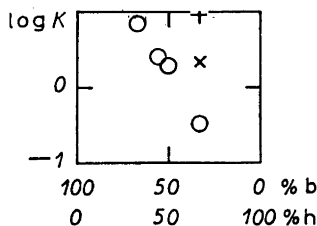


Fig. 2. The influence of the ratio of the volumes of benzene and heptane on the partition coefficient K for *l*-curine (O), and its monomethyl ethers (x and +) between two solvent phases consisting of benzene and heptane in equilibrium with a mixture of equal volumes of methanol and water containing buffering salts (pH about 8).

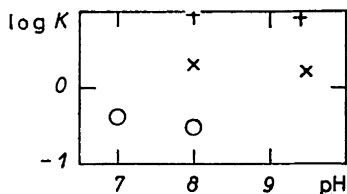


Fig. 3. The influence of pH on the partition coefficient K for *l*-curine (O) and its monomethyl ethers (x and +) between two solvent phases consisting of a mixture of benzene (1/3) and heptane (2/3) in equilibrium with a mixture of equal volumes of methanol and water containing buffer salts (volume ratios 2:4:3).

This solvent system consists of benzene, heptane, methanol, and water, and has proved valuable especially for checking the identity and purity of the alkaloids and the alkaloid derivatives considered here. As part of the procedure for working out separation methods, partition coefficients for *l*-curine and its monomethyl ethers were determined for various compositions of the four component system. The results are given in Figs. 2 and 3. Some preparations used for these measurements were not crystallized. The experiments and the computations were done as described in a previous paper⁴⁰.

It can be seen from Fig. 3 that the influence of pH on the value of the partition coefficient for the two *l*-curine monomethyl ethers is rather small in the four component solvent system as compared to the influence of pH in the case of ether and buffer, whereas the quotient between the partition coefficients has about the same order of magnitude for both solvent systems. For distinguishing between the two *l*-curine monomethyl ethers by determining the partition coefficients it is therefore more convenient to use the four component solvent system than ether and buffer.

Thus, a solvent system consisting of 2 volumes of benzene, 4 volumes of heptane, 3 volumes of buffer pH 8.0 containing sodium carbonate and potassium metabisulfite, and 3 volumes of methanol was usually used for this purpose. The monomethyl ether of *l*-curine which crystallized from benzene or ether had a partition ratio about 9, and the monomethyl ether which crystallized from methanol had a partition ratio slightly less than 3. The partition ratio was calculated as the concentration in the less polar phase (mainly benzene and heptane) divided by the concentration in the more polar phase (mainly methanol and water).

However, upon comparison of *l*-curine and the monomethyl ether of *l*-curine with the partition coefficient most close to that of *l*-curine, it is evident from Figs. 1–3 that in the pH range in which a separation can be carried out conveniently the difference between the logarithms of the partition coefficients is roughly about 10 times larger for ether and buffer than for the four component solvent system. Furthermore, the solubility of *l*-curine and its monomethyl ethers is greater in ether and buffer than in the four component solvent system. Hence, the solvent system ether and buffer is better than the four component solvent system for separating *l*-curine from its monomethyl ethers.

CRYSTALLIZATION OF THE METHYL ETHERS OF *l*-CURINE

The crystallization of bisbenzyltetrahydroisoquinoline alkaloids is greatly influenced by impurities, and purification in the form of ether extraction^{2, 24, 36} is highly advisable. Chloroform, however, has the disadvantage of easily dissolving many of the impurities usually appearing along with these alkaloids.

The *l*-curine dimethyl ether has previously been prepared by several scientists^{11, 34, 36, 38, 43, 44} but they all failed to obtain it in crystalline form. Similarly, *l*-curine diethyl ether seems to have been obtained only as an amorphous substance^{30, 45}. However, during the counter current separation of a large sample of partly methylated *l*-curine, according to a slightly modified^{39, 46} procedure of O'Keeffe *et al.*⁴⁷, *l*-curine dimethyl ether started to crystallize after

a few days from the ether phase in the separatory funnel, from which this substance was then recovered.

As described previously in this paper, a preparation consisting of a mixture of the two *l*-curine monomethyl ethers, but almost completely free from *l*-curine and from the dimethyl ether of *l*-curine, can easily be obtained from partly methylated *l*-curine by two counter current separations, using ether and buffer. One of the components of this monomethyl ether preparation crystallized easily from methanol, but never crystallized from benzene, and it was, as already mentioned, identified as the component with the lower partition coefficient. The other component crystallized from benzene and from diethyl ether but never crystallized from methanol, and it was identified as the component with the higher partition coefficient. It was actually first crystallized in benzene from a preparation obtained by counter current fractionation (with the four component solvent system) of the *l*-curine monomethyl ethers. Crystals usually appeared over night if the mixture was dissolved in either methanol or benzene. During one counter current separation crystals appeared in the ether phase containing *l*-curine monomethyl ethers. Once crystals were obtained, they were used for seeding in subsequent preparations.

On one occasion 90 g of partly methylated *l*-curine was worked up as described. Thereby all available *l*-curine preparations of varying origin and purity were taken. In this case, there appeared as a result of the separation of the monomethyl ether fraction 13 g of the component which crystallized from benzene, and which has the partition ratio 9 in the normally used four component solvent system, and 10 g of the component which crystallizes from methanol and has the partition ratio 3. In addition to this, after the procedure of crystallizing from benzene and from methanol had been run through twice, 0.5 g was obtained of a substance which crystallized from benzene and had the partition ratio 3, and 8 g were recovered of an amorphous residue with the partition ratio 3.

In this case the appearance of three different main components in the monomethyl ether fraction, surprising as it is at first, is, however, what may be expected if an isomer of *l*-curine occasionally occurs in commercial curare material. A similar situation has been described by Dutcher^{48, 49} in the case of tubocurarine and chondrocurarine, *i. e.*, two isomers which, in the normal procedure for preparing tubocurarine, appeared together in the crystallized preparation of tubocurarine. Whereas the distribution of phenolic hydroxyl- and methoxyl groups is the same in tubocurarine and in *l*-curine, no isomer of *l*-curine corresponding to chondrocurarine has yet been reported.

The *l*-curine monomethyl ether with m.p. 206–208°C which Späth and Kuffner³⁸ obtained only in small amounts, was not found in the present investigation, as already mentioned. It cannot be excluded, however, that this substance occurs in the amorphous rest, m.p. 148°C, which once was obtained after two main components of the *l*-curine monomethyl ether fraction had been separated by crystallization. It may be mentioned here that Späth and Kuffner used a preparation of *l*-curine with the m.p. 221–221.5°C, whereas all others have found the m.p. to be about 213°C. Although Späth and Kuffner started with 6 g of curine, they obtained so little of the monomethyl ether with m.p. 206–208°C that only about 3 mg were taken for methoxyl analysis

instead of the usual about 100 mg. This may illustrate the difficulties they encountered in the separation.

Späth and Kuffner's other crystallized *l*-curine monomethyl ether, which crystallized from ether and from methanol, m.p. 154–160°C, and which they considered to still be a mixture, may have mainly consisted of the same *l*-curine monomethyl ether as that obtained in this investigation as a substance crystallizing from methanol, m.p. 148°C, but which was not in this investigation obtained in crystalline form from ether. This may possibly be a case of polymorphism. The differences in m.p. for these compounds are about the same as the differences in m.p. found for curine by Späth *et al.* (221–221.5°C), and by others (212–214°C), respectively.

EXPERIMENTAL

Curine. The *l*-curine used in these experiments was prepared from several lots of curare raw material, originating from Peru, by a method similar to those published in earlier investigations. The melting point of preparations recrystallized from methanol, 213–214°C, agreed well with the values obtained by Böhm^{24, 25} (212°C), by Scholtz^{20, 24, 25, 28, 29} (214°C), by King^{31, 50, 51, 52} (215, 213, and 212°C), by Wieland *et al.*⁵³ (212°C), by Brazil *et al.*⁵⁴ (213–214°C), by Anet *et al.*²⁷ (213–214°C), and by Bhattachari *et al.*⁹ (214°C) but did not agree as well with the value obtained by Späth *et al.*^{36, 38} (221–221.5°C). Kupchan *et al.*⁵⁵ recently for a preparation got m.p. 217–219°C.

pH measurements. The pH values refer to the standard buffer pH = 6.885⁵⁶ and are uncorrected. Sodium salts were avoided in strongly alkaline solutions.

Crystallization tubes. The crystallizations were usually carried out in 5 ml partly conical centrifuge tubes, the tip of the bottom of which had been drawn out to a narrow but not capillary tube about 1 cm long, and sealed. The centrifuge tube, thus transformed into a crystallization tube, was placed in a larger centrifuge tube with a somewhat constricted, ground neck, on the top of which was placed a gasket, cut from a rubber tube of the proper size. The rim of the crystallization tube was just large enough to fit well on the top of the gasket. After the crystallization was finished, the narrow tube in the bottom of the crystallization tube was clipped, and the mother liquor was centrifuged off into the larger centrifuge tube.

Procedure for obtaining crystals from the mixture of monomethyl ethers of curine. One gram of the mixture was dissolved in a conical flask in 5 ml of almost boiling benzene, and insoluble impurities were allowed to settle on the bottom of the flask. The solution was filtered hot through cotton into a crystallization tube. On the following day some wart-shaped crystals of one of the monomethyl ethers had formed. They were recovered and used for seeding in the subsequent separation. — One gram of the mixture of *l*-curine monomethyl ethers was dissolved in 3 ml of almost boiling methanol in a conical flask. On the following day crystals could be recovered. They were subsequently used for seeding.

Procedure for separating the components of the mixture of monomethyl ethers of curine. A solution of the mixed *l*-curine monomethyl ethers in almost boiling benzene was prepared, containing 3 ml of benzene for every gram of solute. The solution was filtered hot through cotton into 5 ml crystallization tubes, and they were immediately seeded with several drops of a benzene suspension of crystals obtained by crushing in a mortar the crystals previously obtained, together with benzene. At least 1 mg of crystals per ml solution should be used. After the crystallization was finished, the narrow tube in the bottom of the crystallization tube was clipped, and the mother liquor was centrifuged off into the larger centrifuge tube. The mother liquor was poured into a Petri dish and frozen and then allowed to evaporate, frozen, in a refrigerator at about 4°C. The substance remaining was dissolved in almost boiling methanol, about 3 ml for every gram of solute. The solution was poured into crystallization tubes and immediately seeded with a few drops of a suspension obtained by crushing the proper crystals together with methanol in a mortar. At least 1 mg of crystals should be used for every ml of solution. After the crystallization

was finished, the crystals and the mother liquor were recovered as described. The mother liquor was poured into a dish, and benzene was added. The solution was partly evaporated and then freeze-dried as described. It was found advantageous to purify the substance remaining after this freeze-drying by extracting it with diethyl ether in a Soxhlet apparatus before it was further worked up. More crystalline substance could be recovered by repeating the procedure with the remainder.

The dimethyl ether of l-curine. This substance crystallized from diethyl ether, m.p. 172°C. It did not change weight on drying *in vacuo* at 65°C. (Found: C 72.6; H 6.8; N 4.6; CH₃O 20.5. Calc. for C₃₈H₄₂O₆N₂: C 72.0; H 6.7; N 4.4; CH₃O 19.5).

The monomethyl ether of l-curine with higher partition ratio. 2.0 g of the crude crystallized compound (crystallized in benzene from the mixture of the two l-curine monomethyl ethers) were dissolved in 17 ml of boiling benzene. The solution was filtered through cotton into crystallization tubes and seeded. 0.7 g was recovered as recrystallized substance, m.p. 142°C; 1.1 g were recovered from the mother liquor. — 1.5 g of the crude crystallized compound were dissolved in dilute hydrochloric acid and thereafter the pH was adjusted to 8.5. The mixture was extracted with about 60 ml of diethyl ether, and the ether phase separated and filtered through cotton. After about 15 min the substance started to crystallize without seeding, both on the glass walls of the container and in the solution. The yield of crystals was 0.4 g, m.p. 132°C; 1.0 g of the substance was recovered from the mother liquor. — 6.9 g of the crude crystallized compound were extracted in a Soxhlet apparatus with about 50 ml of diethyl ether. The weight of the undissolved remainder was 0.3 g. Soon after the extraction was begun, small compact separate crystals started to form on the glass wall of the flask. The yield of crystals was 6.0 g, m.p. 137°C, and 0.7 g were recovered from the mother liquor. — Analysis of the substance crystallized from benzene: C 74.7; H 6.8; N 4.3; CH₃O 14.3; the substance recrystallized from wet ether: C 72.4; H 6.9; N 4.5; CH₃O 17.8; and the substance recrystallized from boiling ether: C 73.0; H 6.6; N 4.6; CH₃O 16.1. Calc. for C₃₇H₄₀O₆N₂·C₆H₆: C 75.2; H 6.8; N 4.1; CH₃O 13.6; calc. for C₃₇H₄₀O₆N₂: C 73.0; H 6.6; N 4.6; CH₃O 15.3.

The monomethyl ether of l-curine with lower partition ratio. 2.0 g of the crude crystallized substance (crystallized in methanol from the residue obtained after one of the l-curine monomethyl ethers had been crystallized from a benzene solution) were dissolved in 19 ml of boiling methanol. The solution was poured into crystallization tubes and seeded. Yield of crystals 1.7 g, m.p. 148°C. Recovered from the mother liquor 0.4 g. (Found: C 72.8; H 6.5; N 4.6; CH₃O 15.3. Calc. for C₃₇H₄₀O₆N₂: C 73.0; H 6.6; N 4.6; CH₃O 15.3.)

Acknowledgements. The author wishes to express his sincere thanks to *Sverige Amerika Stiftelsen*, *American Scandinavian Foundation* and *Astra Pharmaceutical Products, Inc.* for fellowships, and to Professor Dermot B. Taylor for granting the privilege of joining his alkaloid research team and for supplying various *Chondrodendron* alkaloids and partly methylated preparations of them.

REFERENCES

1. Wiggers, A. *Annalen der Pharmacie* **27** (1838) 29.
2. Wiggers, A. *Ann. Chem. Pharm.* **33** (1840) 81.
3. Bödeker, C. *Ann. Chem. Pharm.* **69** (1849) 37.
4. Hanbury, D. *Pharm. J.* [3] **4** (1873) 81, 102.
5. Hanbury, D. *Neues Repertorium für Pharmacie* **23** (1874) 279.
6. Krukoff, B. A. and Moldenke, H. N. *Brittonia* **3** (1938) 1.
7. Flückiger, F. A. *Neues Jahrbuch der Pharmacie* **31** (1869) 257.
8. Flückiger, F. A. *Pharm. J.* [2] **11** (1869) 192.
9. Bhattachari, S., Sharma, V. N. and Dhar, M. L. *J. Sci. Ind. Research (India)* **15B** (1956) 363.
10. Faltis, F. *Monatsh.* **42** (1921) 311.
11. Faltis, F., Kadiera, K. and Doblhammer, F. *Ber.* **69** (1936) 1269.
12. Schlittler, I. E., Heusler, K. and Friedrich, W. *Helv. Chim. Acta* **32** (1949) 2209.
13. Grundon, M. F. *Chem. & Ind. (London)* **1955** 1772.
14. McKennis Jr., H., Hearst, P. J., Drisko, R. W., Roe Jr., T. and Alumbaugh, R. L. *J. Am. Chem. Soc.* **78** (1956) 245.

15. Grundon, M. F. and McGarvey, J. E. B. *J. Chem. Soc.* **1960** 2739.
16. Hager, H. *Handbuch der pharmaceutischen Praxis. Erster Theil*, Julius Springer, Berlin 1876, p. 574—576.
17. *The British Pharmacopoea*, Spottiswoode & Co., London 1885.
18. Hildebrandt, H. *Arch. exp. Pathol. Pharmacol. Naunyn-Schmiedeberg's* **57** (1907) 279.
19. Faltis, F. *Monatsh.* **33** (1912) 873.
20. Scholtz, M. *Arch. Pharm.* **250** (1912) 684.
21. Kondo, H., Satomi, M. and Odera, T. *Ann. Rept. ITSUU Lab.* **4** (1953) 59.
22. Scholtz, M. *Arch. Pharm.* **251** (1913) 136.
23. Husemann, A., Hilger, A. and Husemann, T. *Die Pflanzenstoffe in chemischer, physiologischer, pharmacologischer und toxicologischer Hinsicht*, Julius Springer, Berlin 1882 and 1884, p. 542 and 904.
24. Scholtz, M. *Ber.* **29** (1896) 2054.
25. Scholtz, M. *Arch. Pharm.* **236** (1898) 530.
26. Dutcher, J. D. *J. Am. Chem. Soc.* **68** (1946) 419.
27. Anet, F. A. L., Hughes, G. K. and Ritchie, E. *Australian J. Sci. Research Ser. A.* **3** (1950) 346.
28. Scholtz, M. *Arch. Pharm.* **237** (1899) 199.
29. Scholtz, M. *Arch. Pharm.* **244** (1906) 555.
30. Scholtz, M. *Arch. Pharm.* **249** (1911) 408.
31. King, H. *J. Chem. Soc.* **1940** 737.
32. de Mello Filho, L. E. in Bovet, D., Bovet-Nitti, F. and Marini-Bettolo, G. B. *Curare and Curare-like Agents*, Elsevier, Amsterdam 1959, p. 113.
33. Böhm, R. in Ludwig, C. *Beiträge zur Physiologie*, F. C. W. Vogel, Leipzig 1887, p. 173.
34. Böhm, R. *Abhandl. Kgl. sächs. Ges. Wissensch.* **22** (1895) 199.
35. Böhm, R. *Arch. Pharm.* **235** (1897) 660.
36. Späth, E., Leithe, W. and Ladek, F. *Ber.* **61** (1928) 1698.
37. Scholtz, M. *Pharm. Zentralhalle* **47** (1906) 848.
38. Späth, E. and Kuffner, F. *Ber.* **67** (1934) 55.
39. Hultin, E. *Acta Chem. Scand.* **15** (1961) 1130.
40. Hultin, E. *Acta Chem. Scand.* **15** (1961) 879.
41. Bush, M. T., Goth, A. and Dickison, H. L. *J. Pharmacol. Exptl. Therap.* **84** (1945) 262 footnote 6.
42. Bush, M. T. and Densen, P. M. *Anal. Chem.* **20** (1948) 121.
43. Bick, I. R. C. and Clezy, P. S. *J. Chem. Soc.* **1953** 3893.
44. Kidd, D. A. A. and Walker, J. J. *J. Chem. Soc.* **1954** 669.
45. King, H. *J. Chem. Soc.* **1939** 1157.
46. Jübermann, O. in Müller, E. *Methoden der organischen Chemie* Band I/1, Georg Thieme Verlag, Stuttgart 1958, p. 256.
47. O'Keefe, A. E., Dolliver, M. A. and Stiller, E. T. *J. Am. Chem. Soc.* **71** (1949) 2452.
48. Dutcher, J. D. *Ann. N.Y. Acad. Sci.* **54** (1951) 326.
49. Dutcher, J. D. *J. Am. Chem. Soc.* **74** (1952) 2221.
50. King, H. *J. Chem. Soc.* **1935** 1381.
51. King, H. *J. Chem. Soc.* **1947** 936.
52. King, H. *J. Chem. Soc.* **1948** 1945.
53. Wieland, H., Pistor, H. J. and Bähr, K. *Ann.* **547** (1941) 140.
54. Brazil, O. V., Seba, R. A. and Campos, J. S. *Bol. inst. Vital Brazil* **5** (1945) 79.
55. Kupchan, S. M., Yokoyama, N. and Beal, L. J. *J. Am. Pharm. Assoc.* **49** (1960) 727.
56. Hitchcock, D. I. and Taylor, A. C. *J. Am. Chem. Soc.* **60** (1938) 2710.
57. Hanbury D. and Flückiger, F. A. *Pharmacographica. A history of the principal drugs of vegetable origin, met with in Great Britain and British India*, Macmillan and Co, London 1874, p. 25.
58. Folkers, K. and Unna, K. *Arch. intern. pharmacodynamie* **61** (1939) 370.

Received September 15, 1961.