ACTA CHEMICA SCANDINAVICA 17 (1963) 753–758

Bisbenzyltetrahydroisoquinoline Alkaloids

IV *. Monodemethylated *l*-Curine

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Curine and its methyl ethers are partly demethylated if they are dissolved in concentrated hydrobromic acid or in a mixture of concentrated hydrobromic acid and glacial acetic acid and heated in a steam bath. The crude reaction product is purified by a Soxhlet extraction with ether. Monodemethylated *l*-curine is easily separated from *l*-curine by a counter current procedure using two volumes of ether and one volume of buffer pH 11.0 and a few separatory funnels. Only one of the isomers of monodemethylated *l*-curine has been found. This isomer crystallizes from methanol; m.p. 190–198°C.

One important group of bisbenzyltetrahydroisoquinoline alkaloids has the two benzyltetrahydroisoquinoline rests linked together asymmetrically in two points as shown in Fig. 1. This group of alkaloids comprises tubocurarine, chondrocurine, and curine. A molecule of an alkaloid belonging to this group has four positions for either hydroxyl or methoxyl groups. These four positions are all unequal because of the asymmetry of the molecule. Hence, there are 4 isomers with one hydroxyl group and three methoxyl groups, 6 isomers with two hydroxyl groups and two methoxyl groups, and 4 isomers with three hydroxyl groups and one methoxyl group. Furthermore, there is one derivative with 4 methoxyl groups, and one derivative with 4 hydroxyl groups. In this group of alkaloids there are thus 16 possible derivatives with different combinations of hydroxyl groups and methoxyl groups in four positions. If we take into account that these alkaloids have two asymmetric carbon atoms, so that there are 4 series which may be represented by *d*- and *l*-tubocurarine (the two asymmetric centers unequal) and by *d*- and *l*-curine (the two asymmetric centers equal), the number of substances being discussed amounts to 64.

* Part III. *Acta Chem. Scand.* **16** (1962) 559.

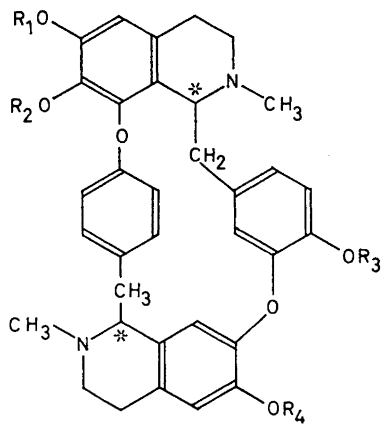

Fig. 1.

	R_1	R_2	R_3	R_4	Centers of asymmetry *
Chondrocurine	H	CH_3	H	CH_3	different
Curine	CH_3	H	H	CH_3	equal
Tubocurarine	CH_3	H	H	CH_3	different

Chondrocurine and curine are tertiary whereas tubocurarine is quaternary.

In connection with the problem of synthesizing bisbenzyltetrahydroisoquinoline alkaloids it is, of course, of interest to learn how these substances can be changed from one to another and how each can be separated from the others. Such a study is also of importance in order that individual substances may become available for pharmacological investigations [1]. The different actions which these substances, as quaternary compounds, may have on the neuromuscular junction, as well as any possible side effects, could then be determined.

Two previous papers [2,3] in this series were concerned mainly with the separation of partly methylated chondrocurine and partly methylated curine, respectively, into three groups of substances: one group with no hydroxyl groups one group with one hydroxyl group, and one group with two hydroxyl groups. In this paper one more group of substances is added, namely, that with three hydroxyl groups. The separations discussed here were carried out with partly demethylated preparations.

Demethylation of *l*-curine and its methyl ethers

Long ago, Scholtz [4] reported the complete demethylation of the methoxyl groups in a bisbenzyltetrahydroisoquinoline alkaloid, now called isochondrodendrine. He made up a solution of the alkaloid in concentrated hydrochloric acid and heated it to 120—130°C for 5 h. The methyl groups attached to the nitrogen atoms of the alkaloid were not removed by this treatment.

In the present work it was considered advantageous to achieve a partial demethylation under athmospheric pressure, and so most of the demethyla-

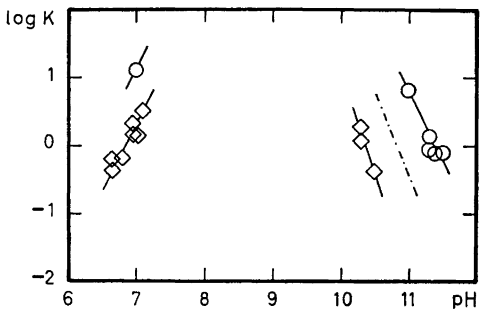

Fig. 2. The influence of pH on the partition coefficient K for *l*-curine (○) and for monodemethylated *l*-curine (◇) between diethyl ether and buffer. The line of dashes and dots gives the optimum relationship between volume ratio and pH for the separation of *l*-curine from monodemethylated *l*-curine.

tions were carried out by heating a solution of the substance in a mixture of glacial acetic acid and concentrated hydrobromic acid in a steam bath under reflux according to Auwers [5] and Stoermer [6]. During this demethylation substances also formed which were insoluble in ether, and it was later found that more of these substances were formed in the presence of acetic acid than in the absence of it.

Separation of *l*-curine and monodemethylated *l*-curine

Only one of the two isomers of monodemethylated *l*-curine was obtained by the procedures described in this paper, and this isomer is for short referred to as the monodemethylated *l*-curine. The other isomer — with two phenolic hydroxyl groups in *ortho* position — has more recently been prepared by another method [7].

In the first attempts to separate the components of preparations of partly demethylated *l*-curine, a counter current procedure was tried on crude preparations, using a buffer solution and either chloroform, a mixture of chloroform and ether, or ether. Thereby emulsions formed, so that the separation procedure, including centrifugation of the mixture, was rather time-consuming and not very satisfactory.

The first chemist working with curine was Wiggers [8]. He found that crude preparations of curine, which he called pelosine, could be efficaciously purified in such a way that the dried substance was extracted with dry ethyl ether. This method was tried on crude preparations of partially demethylated *l*-curine. It turned out that preparations, extracted with ether in a Soxhlet apparatus, could easily be fractionated with a counter current procedure including ether and alkaline buffer as described later on in this paper. If the pH value of the buffer and the relative volumes of ether and buffer were correctly chosen, the *l*-curine was obtained in the ether phase, but the monodemethylated *l*-curine was obtained in the water phase. It appeared as a precipitate when the pH value of the water phase was adjusted to about 8.5. The precipitate obtained in such experiments contained impurities — in spite of the previous

purification — with the capability of forming emulsions if the suspension was extracted with ether. Hence, it was better to filter off the precipitate, dry it, and again extract the substance with ether in a Soxhlet apparatus.

The conditions for separating *l*-curine and monodemethylated *l*-curine were studied in the way described in previous work in this series. The partition coefficients[9,10] of these substances for ether and buffer at various values of pH are given i Fig. 2. It is obvious from this figure that a separation is more efficient on the alkaline side than on the acid side, as the difference between the partition coefficients is larger on the alkaline side. The volume ratio between the two solvent phases which gives the best separation can be read as a function of pH from a line drawn half way between the two lines fitted to the distribution coefficients of the substances[11]. For an actual separation the most suitable volume ratio depends, of course, on the extent to which a sample of *l*-curine may have been demethylated. Thus, if it was demethylated to only a small extent, a relatively larger volume of the ether phase should be taken than if it had been strongly demethylated. The pH value of the buffer is chosen to fit the volume ratio.

The partition ratio of *l*-curine about pH 11 for the solvent pair ether and buffer is a few hundred times larger than that of monodemethylated *l*-curine. Hence, even if the pH value at which a separation is carried out were to differ a few tenth of a unit from the optimum value, the recovery [2] of the two fractions would be expected to be about 99 %, if three separatory funnels were used, and about 99.8 %, if four funnels were used.

A four component solvent system for monodemethylated *l*-curine

In previous papers of this series it was mentioned that a four component solvent system had been used for separating, purifying, and identifying *l*-curine and *d*-chondrocurine, and their methyl ethers. Mixtures of benzene, heptane, methanol, and water containing buffering salts and sulfite were used for that purpose. As to monodemethylated *l*-curine, it is possible to use a similar four component solvent system, containing, however, diethyl ether or chloroform instead of heptane. Thus, the partition coefficient for monodemethylated *l*-curine is about 3 in a mixture consisting of equal parts of benzene, diethyl ether, methanol, and buffer pH 7.

Crystallization of monodemethylated *l*-curine

Monodemethylated *l*-curine can be crystallized from methanol. As methanol is also the solvent of choice for the crystallization of *l*-curine [12,13] and for the crystallization of one of the monomethyl ethers of *l*-curine ,[3] it seems advisable to isolate the monodemethylated *l*-curine by a counter current separation before it is crystallized.

EXPERIMENTAL

Partial demethylation. Fifteen grams of *l*-curine were dissolved in 75 ml of glacial acetic acid, and 50 ml of concentrated hydrobromic acid were added. The mixture was kept in a flask with a condenser and was heated in a steam bath for 2 h, whereupon it was allowed to cool down. About the same volume of water was added, and the mixture was placed in an ice water bath. A cooled 25 % sodium hydroxide solution was slowly added, until the pH value went up to about 8.5. The precipitate which first formed while the sodium hydroxide solution was being added was sticky; after its removal it could be transformed into a non-sticky precipitate by either of two methods: the precipitate could be dissolved in hydrochloric acid and again precipitated with sodium hydroxide, or it could be dissolved in a sodium hydroxide solution and then precipitated with hydrochloric acid. The precipitates were filtered off and dried in a vacuum desiccator over silica gel. Yield 16.8 g of crude preparation.

Purification of the crude preparation of partially demethylated l-*curine.* The preparation was crushed and extracted with diethyl ether in a Soxhlet apparatus. Yield 11.4 g.

Separation of l-*curine from monodemethylated* l-*curine.* A discontinuous counter current separation [2,11] was run with two volumes of ether and one volume of buffer pH 11.0, containing 1 % of $K_2S_2O_5$, 2 % of $Na_2CO_3.H_2O$, 1 % of K_3PO_4 and about 0.3 % KOH. Four separatory funnels were used with 240 ml of the solvent pair in each. About 2 g of the preparation of *l*-curine and monodemethylated *l*-curine were taken each time; altogether 11.4 g. Yield *l*-curine fraction 6.3 g, crude fraction of monodemethylated *l*-curine 4.6 g. The last-mentioned fraction, on extraction with ether in a Soxhlet apparatus, gave 3.2 g of monodemethylated *l*-curine.

Crystallization of monodemethylated l-*curine.* A solution of monodemethylated *l*-curine in hot methanol was prepared, containing about 0.2 g of the substance in 5 ml. The solution was allowed to cool down at room temperature and was then transferred to a cold room (+ 4°C). Crystals soon started to appear; yield 95 mg. Similarly prepared and treated solutions with higher concentrations did not crystallize but the substance came out sticky. 1.0 g of monodemethylated *l*-curine was dissolved in 11 ml of boiling methanol. The solution was poured into crystallization tubes [3,14] and heavily seeded with a suspension of crystals in methanol, ground in a mortar. Crystallization occurred smoothly and the tubes were left in the cold room for a few hours. The mother liquor was centrifuged off. Yield of crystals: 0.7 g; m.p. 190—198°C. The crystals were not quite white but slightly yellow. (Found: C 69.9, H 6.7, N 4.5, CH_3O 6.6; calc. for $C_{35}H_{36}O_6N_2$: C 71.0; H 6.1, N 4.7, CH_3O 5.2.)

Partial demethylation of l-*curine dimethyl ether.* Thirteen grams of *l*-curine dimethyl ether were dissolved in 60 ml of glacial acetic acid, and 40 ml of conc. (47—49 %) hydrobromic acid were added. The mixture was heated in a steam bath for 75 min, and the alkaloid derivatives recovered. The crude preparation was extracted with ether in a Soxhlet apparatus. Yield 11.5 g. This preparation was fractionated as described to give substances with no hydroxyl group, or with one, two or three such groups. Yield: *l*-curine dimethyl ether 1 g, *l*-curine monomethyl ether isomers 4 g, *l*-curine isomers 2.5 g, isomers of monodemethylated *l*-curine 0.28 g.

Partial demethylation of l-*curine with hydrobromic acid.* Five grams of *l*-curine were dissolved in 50 ml of concentrated hydrobromic acid and heated in a steam bath for 2 h. The hydrobromide of monodemethylated *l*-curine started to crystallize during the procedure, and the substance was identified by its partition coefficient.

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 the privilege of joining his alkaloid research team and for supplying various *Chondrodendron* alkaloids and partly methylated preparations of them.

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Received September 17, 1962.