Isolation of Quaternary Bases from *Amanita muscaria* L.

Studies in the Muscarine Series. III*

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Received May 29, 1955

A description is given of the isolation of crude hydrochlorides (200 g) of quaternary bases from 1136 kg. of fly mushroom (*Amanita muscaria* L.). Paper chromatograms of these hydrochlorides, using butanol:water:pyridine (6:3:2) showed, on development with the Levine-Chargaff reagent, six visible spots with the following R_F values: 0.02, 0.09, 0.14, 0.18, 0.24 and 0.31. Preparative chromatography on cellulose columns, with 1.5 N ammonia-saturated n-butanol, yielded the muscarine fraction, which gave after purification muscarine hydrochloride showing R_F 0.26.

In connection with our work on optically active amino aldehydes we started in 1952 with the preparation of optically active compounds of the formula I and II. According to Kögl, one of these compounds must have the structure of muscarine. Kögl's experiments in the synthesis of muscarine failed. He explained the complete physiological inactivity of his synthetic compounds with the great stereospecificity of the muscarine molecule. It was, therefore, of considerable interest to synthesize optically active quaternary ammonium aldehydes.

\[
\begin{align*}
\text{I} & : \text{CH}_3\text{CH}_2\text{CHOH} \cdot \text{CH} \cdot \text{C} = \text{H} \\
\text{II} & : \text{CH}_3\text{CH}_2 \cdot \text{CH} \cdot \text{CHOH} \cdot \text{C} = \text{H}
\end{align*}
\]

We were soon able, in connection with Fourneau's experiments on the synthesis of muscarine-like compounds, to show that the physiologically most active derivatives are not the compounds with free aldehyde groups, but their cyclic acetals.

On the other hand, contrary to Kögl's assumptions, the stereoisomers of simple ammonium aldehydes at least, showed no marked differences in muscarinic activity.

We started, therefore, in 1953, with the isolation of natural muscarine from *A. muscaria* using paper chromatography, countercurrent distribution and ion exchange methods. In the meantime Eugster and Waser proposed a new molecular formula for the muscarine cation, \( \text{C}_9\text{H}_{20}\text{O}_2\text{N}^+ \), with no aldehyde, \( \text{C} = \text{O} \) or any other unsaturated group; therefore muscarine must evidently be a cyclic base.

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* Paper II, see reference⁶
The findings of Eugster and Waser gave once again a new aspect to the already a century old problem of muscarine. The toxic principles of the fly mushroom \((A.\ muscaria\ L.)\) were first described by Schrader (1811), Vauquelin (1813) and Letellier (1826). In their book »Das Muscarin«, Schmiedeberg and Koppe described the isolation and fundamental pharmacological properties of muscarine. The separation of muscarine from choline via the chloroaurate was first described by Harnack, and he proposed the formula \(C_5H_{14}O_2NAuCl_4\). In 1877 Schmiedeberg and Harnack obtained a chloroaurate of the formula \(C_5H_{14}O_2NAuCl_4\) by the oxidation of choline with nitric acid, and claimed muscarine to be identical with betaine aldehyde. This statement can be sometimes found in some textbooks of pharmacology. In 1893 Meyer found that betaine aldehyde in doses of 10 milligrams had no action on frog hearts. In 1912 Ewins found that »synthetic muscarine« obtained from choline and nitric acid was, in fact, choline nitrite \((CH_3)_3N(Cl)CH_2CH_2O \cdot NO\). King isolated muscarine on the basis of its solubility in absolute ethanol, and separated it from choline via the chloroaurate; he came to the conclusion that muscarine must be a more complicated alkaloid base. Kögl, Duisberg and Erxleben isolated muscarine in 1931 as the reineckate, and proposed the formula \(C_8H_{18}O_2N^+\) for muscarine, with the structures I or II. In 1954 Eugster and Waser proposed the formula \(C_9H_{20}O_2N^+\) for muscarine, which they purified by paper chromatography.

King found that the muscarine: choline ratio in the fly mushroom is 1:20, which rendered the purification of muscarine very difficult. The change from Harnack's \(C_5\) molecular formula for muscarine to the newest \(C_9\) formula shows the progress in methods for the separation of chemically similar substances. With regard to the still unanswered question on the structure of muscarine we decided to continue our studies in this series, and in the present paper the course of isolation of muscarine from the fly mushroom \((Amanita muscaria\ L.)\) is described.

In 1953 we collected comparatively small quantities of fly mushroom in beech and oak woods, which are common in the neighbourhood of Zagreb. Because of unsatisfactory results we extended, in 1954, with the aid of the State Department of Forestry, our inquiries to the whole territory of Croatia. However, in beech and oak woods very small quantities of this mushroom could be found; on account of reports on the occurrence of fly mushroom in earlier years, we investigated also young birch woods in the area south-west of Zagreb. It may be of interest to note that in these birch woods fly mushroom was found in great quantities.

For the benefit of chemists for whom it may become necessary to collect larger quantities of \(Basidiomycetes\), we would like to draw the attention to the fact that certain fungi are restricted to the area beneath particular species of trees and are never found in significant quantities under other kinds of trees. Many species of \(Basidiomycetes\) form mycorrhizae with trees. It is further known that the fly mushroom forms mycorrhizae with the birch \((Betula alba)\), larch \((Larix decidua)\) and other trees. In our case we found most of our crop of fly mushroom in woods which consisted mainly of birch \((Betula pendula)\), with some alder \((Alnus glutinosa)\).

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\(^a\) Earlier data on the isolation and characterization of muscarine can be found in Kögl's first paper on muscarine.

and hornbeam (Carpinus betulus), under juniper bushes (Juniperus communis), and with certain mosses, especially Scleropodium purum, and Polytrichum atenuatum. Over one thousand kilograms of fresh fungi were collected, homogenized, extracted with ethanol, and the aqueous-ethanolic extract evaporated in vacuo. Further purification was carried out using King's findings, who established the fact that muscarine is soluble and stable in absolute ethanol. In this manner, by precipitation of the aqueous ethanolic extract with absolute ethanol, filtrates were obtained which showed 4500 Muscarine Units per gram of dry residue, while the dry residue of the crude aqueous ethanolic extract showed only 300–330 Muscarine Units per gram. Further concentration was carried out by precipitation with ammonium reineckate solution, and in this manner we obtained 750 g. of crude reineckates of choline, muscarine and other quaternary bases. These reineckates were converted to hydrochlorides following Kapfhammer, and showed 30000 Muscarine Units per gram of hydrochlorides. In this manner 200 g. of hydrochlorides were obtained as a partly crystallized solid. We attempted the Craig countercurrent distribution of crude reineckates in the solvent system acetone-ethyl acetate-diethyl ether-water. In 100 transfers it was impossible, in this solvent system, to separate muscarine from choline in any significant degree.

Preliminary experiments on paper chromatography of the crude hydrochlorides proved that the solvent systems butanol-water-pyridine, and butanol-ammonia are the most suitable for this kind of work. The quaternary bases were best detected with the Levine-Chargaff reagent. In the case of butanol-water-pyridine six spots appeared with R_f values 0.02, 0.09, 0.14, 0.18, 0.24, and 0.31. The biologically most active sections of the paper chromatogram were those between R_f 0.19 and 0.29; the spot with R_f 0.14 is due to choline. In the solvent system n-butanol-ammonia we obtained spots with R_f values 0.11 (choline), 0.16, 0.31, 0.62 and 0.76. Throughout the experimental work on paper chromatography we used the solvent system butanol-water-pyridine, and for preparative paper chromatography on cellulose columns we preferred the use of the butanol-ammonia solvent as mobile phase. On columns prepared from cellulose powder according to Jones and coworkers, it was possible to separate the main quantity of muscarine from choline hydrochlorides. We used the 200-fold quantity of cellulose powder for the separation of the hydrochlorides of quaternary bases.

According to the work of Harnack and King, we converted this crude muscarine concentrate into the chloroaurates. Fractional crystallization of these chloroaurates gave a slightly soluble chloroaurate, m. p. 220°, which converted to the hydrochloride according to Dudley, gave only one spot on the paper chromatogram, R_f 0.19. From the mother liquors we obtained the crude chloroaurate of muscarine in the form of pale yellow leaflets, which after conversion into the hydrochloride showed an intensive spot on the paper chromatogram, R_f 0.255 ± 0.005, and very slight spots with R_f values 0.19 and 0.35. Chromatography on a cellulose column of this hydrochloride gave pure muscarine chloroaurate, m. p. 117.5–118°.

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c We are indebted for these botanical determinations to Dr. R. Domac from the Department of Botany, Faculty of Science, Zagreb.
**Acknowledgments.** We wish to thank the Fund for Scientific Research of the University of Zagreb for financial assistance during 1954—1955. We are, further, indebted to Professor A. Stampar, Director of the School of Public Health, Zagreb, for facilities in the collecting of biological material, to Mr. V. Stetić of the State Department of Forestry, Zagreb, for help in the organization of the collecting, and to the Institute «Rudjer Bošković» for several fellowships (B. G., T. G., S. G.). We would also like to express our thanks to the staff of the Chemical Institute for help in the earlier stages of this work.

**EXPERIMENTAL**

**Preparation of the Aqueous-ethanolic Extract of A. muscaria**

(with V. Tomasić, A. Labavici and S. Ghyczy)

Fresh fly mushrooms (1136 kg.) were thoroughly cleaned and homogenized immediately after collecting (not later than 12—24 hours) in a Waring blender with an equal quantity of ethanol. This mixture was put into containers and immediately stored in a cold room at —5° for a week. Ethanol was then added with stirring until the total quantity of ethanol used was 2450 l. The supernatant liquid was decanted, filtered, and the fungi thoroughly pressed in a tincture press. The combined aqueous-ethanolic extracts were evaporated under 20° to a volume of 200 l. This concentrate contained, on the average, 17—20% of dry residue which showed an activity of 300—330 Muscarine Units per gram. The volume of the extract was reduced, under the same conditions, to 53 l., and the concentrate thus obtained contained 51% of dry residue. The concentrate was slowly poured into 106 l. of absolute ethanol and left at 0° for 24 hours. The supernatant liquid was siphoned off and evaporated in vacuo to a volume of 20°, until the concentrate contained 31—33% of dry residue. This concentrate was evaporated with 5 l. of peroxide-free ether. The aqueous layer (38 l.) was again poured, with vigorous stirring, into 60 l. of absolute ethanol and left at —5° overnight. The supernatant liquid was again siphoned off and evaporated to a volume of 13.6 l., the concentrate containing 50% of dry residue. The concentrate showed an activity of 4500 Muscarine Units per gram of dry residue. It was shaken with 4 X 4 l. of ether, and the combined extracts washed with 1 l. of water; the combined aqueous layers had a volume of 13.6 l. (Extract A). The pressed fungi from the tincture press were homogenized in an equal quantity of 60% ethanol, with mechanical stirring. The resulting aqueous-ethanolic filtrate was treated in the same manner as described for the preparation of Extract A.

**Preparation of Reineckates (with V. Jagodic)**

To extract A (12 l., 55% of dry residue) a 3% aqueous ammonium reineckate solution (20 l.) was added with vigorous stirring. The mixture was left at 0° for 24 hours, and then the precipitate filtered off and dried in vacuo at 25—30°; dry reineckates (750 g.) were obtained.

**Craig Countercurrent Distribution of Crude Reineckates**

(with R. Bevec and V. Skaric)

The distribution was carried out in a 200-tube modification of Craig’s all-glass apparatus at 21°, using 205 mg. of crude reinecke salt, in the solvent system acetone-ethyl acetate-diethyl ether-water (1:1:1:2, by volume), and in 101 transfers. The solvents were purified and distilled before use and equilibrated in separatory funnels. In preparing the machine for fractionation, 25 ml. of the lower phase were added to each of the tubes. The substance was dissolved in the lower phase and added to tubes 4 and 5. About five transfers were made per hour. All the tube fractions were concentrated to dryness by vacuum distillation. The contents of each tube were converted to hydrochlorides according to Kapfhammer (vide infra). The biological activity was distributed between the contents of tubes 36—54. The maximal biological activity was shown by the contents of tube 46. In practically the same
range (tubes 33–59) choline reineckate was distributed (a separate experiment was carried out with synthetic choline reineckate); it is, therefore, impossible to separate muscarine from choline in the reineckate form in the solvent system used.

Conversion of Reineckates to Hydrochlorides

Thoroughly dried and finely powdered reineckates (50 g.) were dissolved in acetone (1000 ml.). The undissolved residue was filtered off (23%), the filtrate was diluted with water (200 ml.) and treated according to Kapfhammer17 with a silver sulphate solution (15 g. of Ag₂SO₄ in 2.5 l. of water). This solution was added drop-wise and with vigorous stirring. The mixture was left overnight at 0°. The precipitated silver reineckate was centrifuged off and the supernatant liquid treated with the equivalent quantity of barium chloride solution (11.79 g. of BaCl₂ · 2H₂O in 1.15 l. of water). The precipitated barium sulphate was centrifuged, the supernatant liquid evaporated to dryness in vacuo below 20° in an atmosphere of nitrogen. The residue was dissolved in absolute ethanol, filtered, and again evaporated in vacuo below 20°. The obtained mixture of hydrochlorides showed an activity of 30000 Muscarine Units per gram. From 50 g. of reineckates 13.3 g. of hydrochlorides were obtained. The whole quantity of reineckates was converted to the hydrochlorides in 100 g. batches, and 200.5 g. of hydrochlorides were obtained as a crystalline solid.

Paper Chromatography of Crude Hydrochlorides

Paper chromatography was carried out on Whatman No. 1 paper, at 20°, during 24 hours, with butanol-water-pyridine (6:3:2) as mobile phase. The quaternary bases were detected with the Levine-Chargaff reagent18, d). On the developed chromatogram of the crude hydrochlorides six spots appeared with Rₖ values 0.02, 0.09, 0.14, 0.18, 0.24 and 0.31. Undeveloped chromatograms were cut into sections, every section eluted, and the eluates biologically tested for muscarinic activity; the most active section was between Rₖ 0.19–0.29. On undeveloped chromatograms a green fluorescent spot appeared, with Rₖ 0.21 (ultraviolet lamp.). Comparison with synthetic material showed that the spot with Rₖ 0.14 was due to choline.

Chromatography of Crude Hydrochlorides on Cellulose Columns

a) Chromatography with the solvent system butanol-water-pyridine (6:3:2) (Solvent A)

The column of powdered cellulose was prepared according to Hough, Jones and Wadman21. With 500 g. of Whatman Cellulose Powder, B Quality, Standard Grade, a column of 49 × 4 cm. was prepared. When the column is being packed great care must be taken to ensure that that the packing is uniform, otherwise a subsequent distortion of the zones of quaternary bases will result. The cellulose powder is introduced into the glass tube in portions sufficient to fill only one centimetre of its length. After each addition, the cellulose powder is packed by tapping the base of the glass tube gently and repeatedly on a piece of wood. It is then further uniformly compressed with a suitable wooden rod fitted with a plunger which has at least half the diameter of the column. The surface of the cellulose column must be flat and horizontal in order to avoid distortion of the zones, and it is covered with a thick filter paper disc to prevent the surface from being disturbed. The column is tightly packed in this manner, and treated before use with 1 g. 8-hydroxyquinoline in 25 ml. of solvent A, and washed with 2 liters of the same solvent until all the soluble impurities are removed. Once the column has been washed it should always remain covered with a 4 cm. layer of solvent.

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d Using Draggendorf’s reagent19 under the same conditions, only the spot due to choline appeared (Rₖ 0.14).

e All biological tests were carried out on isolated frog hearts (Rana esculenta) following the technique of Kögl et al.2
Table I.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Muscarine units/mg.</th>
<th>R_F values Solvent A, Levine-Chargaff reagent^{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—50</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>51—120</td>
<td>—</td>
<td>0.54</td>
</tr>
<tr>
<td>121</td>
<td>—</td>
<td>0.18 0.23 0.25</td>
</tr>
<tr>
<td>130</td>
<td>370</td>
<td>0.18 0.24 0.25</td>
</tr>
<tr>
<td>140</td>
<td>73000</td>
<td>0.18 0.23 0.26</td>
</tr>
<tr>
<td>150</td>
<td>158000</td>
<td>0.19 0.23 0.26</td>
</tr>
<tr>
<td>160</td>
<td>672000</td>
<td>0.18 0.24 0.26</td>
</tr>
<tr>
<td>165</td>
<td>1200</td>
<td>0.14 0.19 0.23 0.33</td>
</tr>
<tr>
<td>296</td>
<td>—</td>
<td>0.1 0.14 0.31</td>
</tr>
</tbody>
</table>

The crude hydrochlorides (5 g.) were dissolved in solvent A (50 ml.) and mixed with 10 g. of cellulose powder. This slurry was poured onto the cellulose column and uniformly mixed with the solvent on top of the column. The eluate was fractionated into approximately 10-ml. portions by an automatic device which changed the receiver after the appropriate interval of time (20 minutes). 300 fractions were collected. Each fraction was evaporated to dryness, weighed and characterized by paper chromatography and biological test. The biological activity was distributed between fractions 121—300. Elution of choline began in fraction 165 (Table I).

The fractions with the same R_F values were combined and evaporated to dryness in vacuo below 250.

fractions                      dry residue
(a) 1—50                       143 mg.
(b) 51—120                     632 mg.
(c) 121—164 (muscarine)        490 mg.
(d) 165—300 (choline)          3325 mg.

The cellulose column was washed with 500 ml. of solvent A and after evaporation 388 mg. of dry residue were obtained (fraction e). The total weight of fractions a—e was 4.978 g.

Table II.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Muscarine units/mg.</th>
<th>R_F values Solvent A, Levine-Chargaff reagent^{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>31—60</td>
<td>—</td>
<td>0.31 0.50</td>
</tr>
<tr>
<td>61—110</td>
<td>—</td>
<td>0.31</td>
</tr>
<tr>
<td>120</td>
<td>390</td>
<td>0.31</td>
</tr>
<tr>
<td>150</td>
<td>642000</td>
<td>0.20 0.26 0.30</td>
</tr>
<tr>
<td>190</td>
<td>40000</td>
<td>0.20 0.26 0.30</td>
</tr>
<tr>
<td>195</td>
<td>2000</td>
<td>0.14 (choline)</td>
</tr>
<tr>
<td>200—550</td>
<td>—</td>
<td>0.14</td>
</tr>
</tbody>
</table>
b) Chromatography with the solvent system butanol-ammonia (Solvent B)

As solvent system, ammonia-saturated n-butanol was used (4 parts n-butanol saturated with 1 part of 1.5 N aqueous ammonia). Preliminary experiments in paper chromatography with this solvent showed that the separation of crude hydrochlorides is better than with solvent A. With Whatman No. 1 paper, at 20°, we obtained spots with Rf values 0.11 (= choline), 0.16, 0.31, 0.62 and 0.76. The cellulose column was prepared as described above, with Whatman Ashless Cellulose Powder, Standard Grade, but using solvent B, and 2.5 g. of crude hydrochlorides. Five hundred and fifty 15 ml.-fractions were collected (Table II).

The fractions with the same Rf values were combined and evaporated to dryness in vacuo below 25°.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 1—35</td>
<td>102</td>
</tr>
<tr>
<td>(b) 36—139</td>
<td>591</td>
</tr>
<tr>
<td>(c) 140—194</td>
<td>220</td>
</tr>
<tr>
<td>(d) 195—350</td>
<td>902</td>
</tr>
</tbody>
</table>

The cellulose column was washed with solvent B until the sample of effluent gave no reaction on quaternary bases (paper chromatography). After evaporation 634 mg. of dry residue were obtained (fraction e). The total weight of fractions a—e was 2,450 mg.

For the separation of larger quantities of hydrochlorides (15 g.) we used columns of Whatman Cellulose Powder, Ashless, Standard Grade (3 kg.), of the dimensions 10 X 78 cm.; fractions of 100 ml. were collected in time intervals of 20 minutes.

Preparation of Chloroaaurates from the Muscarine Fractions (c) Obtained by Chromatography with solvent A

Chloroaaurates were prepared according to King13. Fractions 121—164 (c) obtained by chromatography with solvent A, having 490 mg. of dry residue, showed 340000 Muscarine Units/mg. Paper chromatography of the residue (200 g.) gave, using Whatman No. 1 paper, and solvent A, the Rf values 0.23, 0.25, 0.31 and 0.46. The residue (490 mg.) was dissolved in 1% hydrochloric acid (2.5 ml.) and treated with a slight excess of 10% gold chloride. The precipitated chloroaaurates were fractionated, and a slightly soluble chloroaurate of the constant m. p. 220° obtained. From the mother liquor, another chloroaurate was obtained by further fractional crystallization as pale yellow leaflets, m. p. 111—112°, which corresponded to the chloroaurate described by King13. By converting this chloroaurate into the hydrochloride following Dudley22, a most active muscarine concentrate was obtained. Paper chromatography of this concentrate (Whatman paper No. 1, solvent A, 20°) showed an intensive spot, Rf 0.255 (± 0.005), and very slight spots with Rf values 0.19 and 0.35.

The chloroaurate of the m. p. 220° converted to the hydrochloride in the same manner showed on the paper chromatogram only one spot, Rf 0.19. This hydrochloride shows no biological activity.

Preparation of Chloroaaurates from Muscarine Fraction (c) Obtained by Chromatography with Solvent B

Fractions 140—194 (c) obtained by chromatography with solvent B, yielding 220 mg. of dry residue, were combined and dissolved in 1% hydrochloric acid (2.5 ml.) and treated with a slight excess of gold chloride. Further treatment was carried out as described above. The hydrochlorides obtained from crude muscarine chloroaaurate showed on the paper chromatogram an intensive spot with Rf 0.26 and two weak spots with Rf 0.19 and 0.23. From the mother liquor of the chloroaaurates, hydrochlorides were obtained which showed on the paper chromatogram only one spot, Rf 0.19.
Purification of Crude Muscarine Hydrochloride

Fractions (c) of crude muscarine hydrochloride obtained by chromatography (500 mg. of dry residue) were fractionated on a column of Whatman Cellulose Powder Ashless, Standard Grade, (100 g.), of the dimensions 2.4 × 42 cm.; solvent B was used and fractions of 1.5 ml. were collected in 20 minute intervals. Fractions 60—80 showed only one spot on the paper chromatogram, R_F 0.26, due to pure muscarine hydrochloride, which converted to the chloroaurate showed the m. p. 117.5—118°C.

<table>
<thead>
<tr>
<th>Tube number</th>
<th>Muscarine units/mg.</th>
<th>R_F values Solvent B, Levine-Chargaff reagent\textsuperscript{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5—10</td>
<td>—</td>
<td>0.14</td>
</tr>
<tr>
<td>15</td>
<td>3200</td>
<td>0.08 0.14 0.23</td>
</tr>
<tr>
<td>20</td>
<td>7000</td>
<td>0.08 0.14 0.23</td>
</tr>
<tr>
<td>25</td>
<td>10000</td>
<td>0.23 0.30</td>
</tr>
<tr>
<td>30</td>
<td>4000</td>
<td>—</td>
</tr>
<tr>
<td>35—130</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>135—150</td>
<td>—</td>
<td>0.18 0.27</td>
</tr>
<tr>
<td>155—200</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

TABLE III.

Fig. 1. Craig Countercurrent Distribution. Solvent system n-butanol-1.5 N ammonia (4:1), 200 transfers, 2.5 g. of hydrochlorides of quaternary bases. Plot: dry residue weight in milligrams of 10 ml. lower and 10 ml. upper phase versus tube number. Dotted line: Muscarine Units in arbitrary scale.

Craig Countercurrent Distribution of Crude Hydrochlorides
(with R. Bevec and V. Škarić)

The distribution was carried out in the solvent system B (4 parts of n-butanol saturated with 1 part of 1.5 N aqueous ammonia), and in 200 transfers, with 25 ml.
of the lower, and 25 ml. of the upper phase. The crude hydrochlorides (2.5 g.) were dissolved in the lower phase and added to tubes 4–9. About five transfers were made per hour. The tube fractions were concentrated to dryness by vacuum distillation and the dry residue subjected to paper chromatography (2-00 sample) and biological tests. The results are given in Fig. 1. Choline hydrochloride was distributed in tubes 5–25. The biological activity was the greatest in tubes 15–30. The weight of dry residue of the contents of tubes 1–35 was 2.17 g (cf. Table III).

**Attempted Separation of Quaternary Bases on Cation Exchangers**

*(with Z. Stefanac)*

*a) Experiments with Amberlite IRC-50f.*

The resin used was in sodium form, 16–50 mesh, 1 g., 4 × 1.2 cm. column, with a maximum weight capacity of 10.5 milliequivalents per dry gram, and the flow rate was 12 ml/hr. A one per thousand solution of choline hydrochloride was filtered through the column and eluted with m/15 phosphate and m/5 acetate buffer. Elution of choline was determined in the filtrate with KJ + J reagent. The hydrochlorides of crude quaternary bases were also used in one per thousand solutions; choline was determined as described above, and muscarine with biological tests. It was shown that elution of choline takes place in the range between pH 4.80–7.64; the elution of muscarine occurred also in the same range.

*b) Experiments with Ionac C-100g.*

The exchanger used was in sodium form, 4 × 1.2 cm. column, and the flow rate was 12 ml/hr. The experiments were performed as in a). Between pH 4.60–5.64 elution of choline was determined. Muscarine hydrochloride was eluted in practically the same range, and therefore it was impossible to separate muscarine from choline hydrochlorides under the described conditions.

**REFERENCES**

6. K. Balenović and coworkers, unpublished experiments.

f Rohm & Haas Co., Philadelphia 5, Pa., U.S.A.
9 The Ionac Co., New York 36, N. Y., U. S. A.
IZVOD

Izolacija kvaternih baza iz Amanita muscaria L.

3. Priopćenje o muskarinu

K. Balenović, D. Cerar, B. Gašpert i T. Galijan

Opisuje se dobivanje hidroklorida kvaternih baza (200 g) iz 1136 kg muhare (Amanita muscaria L.). Papirni kromatogrami ovih hidroklorida pokazali su — pri upotrebì otapala butanol:voda:piridin (6:3:2), nakon razvijanja reagensom Levine-Chargaff — šest vidljivih mrlja sa \( R_f \) vrijednostima 0.02, 0.09, 0.14, 0.18, 0.24 i 0.31. Preparativnom kromatografijom dobivena je na stupcima od celuloznog praška, s butanol-amonijakom (butanol zasićen 1.5 N amonijakom), muskarinska frakcija, koja je nakon čišćenja dala muskarin hidroklorid, sa \( R_f \) 0.26.