Studies on the Effect of Marine Products on Cholesterol Metabolism in Rats—XI

Isolation of a New Betaine, Ulvaline, from a Green Laver Monostroma nitidum and Its Depressing Effect on Plasma Cholesterol Levels

Shigenobu Abe* and Takashi Kaneda*

(Received February 18, 1975)

A new betaine, ulvaline, was isolated as a picrate from a green laver Monostroma nitidum and named after its order name Ulvales. Subsequent chemical studies of ulvaline revealed the structure of 3-O-(2,3-dihydroxy-n-propyl)-1-carboxy-n-propyl trimethyl ammonium (O-1-glyceryl homoserine betaine). In the feeding experiment, ulvaline was proved to be a slightly hypocholesterolemic agent.

In a previous paper1, an unknown betaine was isolated as picrate from a basic fraction of water-extract of green laver which reduced plasma cholesterol levels. Subsequent work2 led to identification of homoserine betaine in the hydrolyzate of the unknown betaine.

Further efforts to identify the hydrolysis-products resulted in identification of glycerol as the counterpart of homoserine betaine. It was concluded for the results of several analyses, that the unknown betaine was regarded as an ether of homoserine betaine with glycerol. Because of the unique chemical structure and the wide distribution in Ulvales, it was named ulvaline.

This paper deals with chemical structure and feeding experiment of ulvaline.

Experimental and Results

Isolation of ulvaline picrate A basic fraction (F)1 (3.66 g) of water-extract of green laver was dissolved in a small amount of 2.4 N hydrochloric acid and the solution was put on a column of Dowex 50-X8 (H+ form, 200–400 mesh, 2.4×40 cm). The column was developed with 2.4 N hydrochloric acid (1 l) and the eluate was collected in 20 ml-portions. Each fraction was examined for ulvaline by means of thin layer chromatography (Cellulose powder MN 300). Fractions (Nos. 21–27) containing ulvaline were combined and concentrated. The concentrate was passed through a column of Dowex 1-X8 (OH− form, 200–400 mesh, 1.0×20 cm) and the column was washed with water (100 ml). The effluent and washings were combined and evaporated to dryness. The residue was dissolved in 20 ml of 50% ethanol containing 3 g of picric acid and the solution was left overnight.

* Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Sendai (阿部重信・金田慎志： 東北大学農学部食糧化学科)
at room temperature. The resulting picrate was recrystallized twice from 50% ethanol. Yellow needles were obtained, m. p. 137–138°C. Anal. Found: C, 41.21; H, 5.25; N, 12.66%. Calcd. for C₁₅H₂₁O₅N·C₆H₃O₃N: C, 41.38; H, 5.21; N, 12.07%. The infrared absorption spectrum is shown in Fig. 1.

Fig. 1. Infrared absorption spectrum of ulvaline picrate (in KBr).

Preparation of free ulvaline The picrate of ulvaline (500 mg) was dissolved in 50% acetone (10 ml) and percolated through a column of Dowex 1–X8 (OH⁻ form, 200–400 mesh, 1.0 × 5 cm), and the column was rinsed with water (10 ml). The effluent and washings were combined and evaporated to give free ulvaline. The ORD was measured in N HCl (c=0.593) by ORD instrument of JASCO model ORD/UV-5: Positive Cotton effect curve (peak, 235 nm); [ϕ]_{235}^D = +929, [ϕ]_{504}^D = +504, [ϕ]_{500}^D = +34, [ϕ]_{800}^D = 0.

Chemical properties of ulvaline Ulvaline is detectable as a characteristic yellow spot on the chromatogram when sprayed Dragendorff reagent. Table 1 shows its Rf values together with those of the related compounds on paper chromatograms.

Table 1. Rf values of ulvaline and related compounds on paper chromatograms

<table>
<thead>
<tr>
<th>Materials</th>
<th>Solvent</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Ulvaline*¹</td>
<td>0.39</td>
<td>0.49</td>
</tr>
<tr>
<td>Choline*¹</td>
<td>0.52</td>
<td>0.35</td>
</tr>
<tr>
<td>Glycine betaine*¹</td>
<td>0.43</td>
<td>0.47</td>
</tr>
<tr>
<td>Homoserine betaine*¹</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>Isolated</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>Authentic</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>Glycerol*²</td>
<td>0.49</td>
<td>0.72</td>
</tr>
<tr>
<td>Isolated</td>
<td>0.49</td>
<td>0.72</td>
</tr>
<tr>
<td>Authentic</td>
<td>0.49</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Filter paper: Toyo Roshi No. 50.
Solvent systems: A...n-Butanol, acetic acid, water (12: 3: 5).
B...n-Propanol, 2.5 N ammonium hydroxide (7:3).
Color reagents: *¹ Dragendorff reagent.
*² Potassium periodate and benzidine hydrochloride.

The positive reaction with the Dragendorff reagent and the negative one with citric acid and acetic anhydride reagent³ indicated that ulvaline was presumably possessed of
a quaternary ammonium group. A singlet (δ 3.17, 9H) measured in the PMR spectrum (in D₂O) confirmed the presence of N-trimethyl group.

Upon oxidation with periodate, ulvaline gave a substance which changed a decolorized fucsin solution to red color⁴. Therefore, it should be a vicinal polyalcohol.

Ulvaline (0.2 mM) was dissolved in 2 ml of 0.1 N HCl and the solution was titrated with N sodium hydroxide solution in the usual way. The obtained titration-curve agreed very closely with that of glycine betaine (pK₁ᵣ = 1.34, pK₂ᵣ = 14)⁵.

**Hydrolysis of ulvaline** Ulvaline (500 mg) dissolved in 5 ml of 6 N hydrochloric acid was heated in a sealed glass tube for 24 hr at 100–110°C. After reaction, insoluble matters were filtered off and the filtrate was subjected to evaporation under reduced pressure to remove hydrochloric acid. The residue was dissolved in a small amount of water and the solution was applied to a column of Dowex 50-X8 (H⁺ form, 200–400 mesh, 1.0 × 10 cm). Successively the column was washed with 10 ml of water. The effluent and washings were combined and evaporated to dryness (neutral substance, 60 mg). The washed column was developed with 2 N hydrochloric acid. The undecomposed ulvaline (300 mg) appeared in the first 15 ml-portion. Homoserine betaine was eluted in the next 10 ml-portion.

Subtraction of the chemical formula of homoserine betaine (C₇H₁₅O₃N) from that of ulvaline (C₁₀H₂₁O₅N) led to the empirical formula, C₃H₈O₃ (+H₂O), which corresponds to glycerol. Comparison by paper chromatography revealed that the neutral substance was identical with the authentic glycerol (Table 1).

**Feeding experiment** Ulvaline and synthesized homoserine betaine² were tested for hypocholesterolemic activity by our routine method¹. The experimental supplement (0.01–0.10%) was dissolved in 10 ml of water and sprayed onto the control diet (II) containing 1% cholesterol with thorough mixing. After a 18-day feeding period, the rats were killed and the plasma cholesterol was determined by our modified method¹ of SPERRY and WEBB.

As shown in Table 2, slight decreases in the plasma cholesterol levels of two groups of rats administered 0.01 and 0.10% ulvaline are recognizable. On the other hand, homoserine betaine was not effective at all.

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Weight gain (g)</th>
<th>Plasma cholesterol</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Cholesterol-free (basal)</td>
<td>99±5*</td>
<td>62±2*</td>
<td>17±1*</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>II. Cholesterol diet (control)</td>
<td>102±3</td>
<td>149±8</td>
<td>38±2</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>III. Ulvaline 0.10%</td>
<td>100±5</td>
<td>132±7</td>
<td>33±2</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>IV. Ulvaline 0.01%</td>
<td>103±3</td>
<td>133±9</td>
<td>33±2</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>V. Homoserine betaine 0.10%</td>
<td>99±3</td>
<td>165±10</td>
<td>42±3</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

* Mean values of 6 rats±SE.
Ulvaline was detected as a yellow spot on the chromatogram with the Dragendorff reagent, and the color was quite different from those of the ordinary quarternary ammonium compounds such as betaines, choline and choline derivatives. However, it was rather complicated to detect the spot on the chromatograms in the presence of other Dragendorff-positive compounds having similar Rf value. This difficulty makes it necessary for detection of ulvaline to carry out chromatographic separation with cation-exchange resin prior to paper chromatography.

After reaction with periodate, ulvaline became detectable on the chromatograms with the benzidine reagent which usually is employed for sugars and sugar alcohols. Hydrolysis of ulvaline gave rise to homoserine betaine and glycerol. Its reluctance to acid-hydrolysis in a mild condition indicated that the two components were bonded on the basis of an ether-linkage rather than an ester-linkage. And a fair agreement of titration-curve of ulvaline with that of glycine betaine suggested that the carboxyl group of ulvaline was free.

Judging from the positive reaction with the periodate reagent, ulvaline can be possessed of vic-diol. Therefore, it is clear that homoserine betaine links with the terminal carbon of glycerol.

From these findings, the chemical structure of ulvaline was concluded to be 3-O-(2,3-dihydroxy-n-propyl)-1-carboxy-n-propyl trimethyl ammonium (O-1-glyceryl homoserine betaine).

\[
\begin{align*}
\text{H}_3\text{C} \cdot \text{O} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{COO}^- \\
\text{HC} \cdot \text{OH} \\
\text{H}_3\text{C} \cdot \text{OH} \\
\text{N}^+ \\
\text{(CH}_3)_3
\end{align*}
\]

The ORD curve of ulvaline showed the positive Cotton effect but no activity of optical dispersion in the range from 400 to 700 nm. The above data suggests that ulvaline may be induced from a L-series-amino acid. However, configuration at two asymmetric carbons needs further stereochemical studies. The conclusive structure and configuration of ulvaline should be proved by synthesis.

Quite recently, Brown and Elorson have just isolated a novel lipid from phytoflagellate Ochromonas danica and characterized as 1(3), 2-diacylglycerol-3(1)-o-4'-(N,N,N-trimethyl) homoserine\textsuperscript{7).} According to their paper, ulvaline would be considered to be an hydrophilic moiety of the novel lipid. However, the similarity of these compounds needs to be investigated further.

A slight activity of ulvaline was observed for the cholesterol-depressing effect in plasma of rats. But this activity seemed to be less than that of \(\beta\)-homobetaine\textsuperscript{11} which was previously isolated as a hypocholesterolemic constituent from the same layer.
References

4) E. Feigl: ibid., p. 317.