Studies on Amino-acids and Related Compounds. XVI. Formation of *l*-Methionine-Sulphone and β-Methylsulphone-Propionic Acid from *l*-Methionine by Electrolysis.

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The electrolytic oxidation of crude leucine was carried out in the same manner as described in Part XI,(1) and hydrogen sulphide, β-methyl-sulphone-propionic acid, and isovaleraldehyde as well as isovaleric acid were obtained. The formation of these sulphur-containing compounds might be ascribed to methionine. Moreover, the elucidation of the mechanism of the exidation of methionine seemed to be important from the view-point of biochemistry. On these considerations, the electrolysis of methionine was studied and the results are described in the present paper.

Butz and Vignend (1932)(2) obtained homocystine and a little dimethyl disulphide from methionine by heating in 18N-sulphuric acid at 125–135° for 8 hours. Toennies and Kolb (1938)(3) isolated methionine-sulphoxide from *dl*-methionine by its oxidation with hydrogen peroxide. Observing the formation of methyl mercaptan from *l*-thethionine by heating with barium hydroxide and water at 160° under 100 pounds pressure for 5 hours, Onitake (1938)(4) pointed out that the formation of methyl mercaptan from protein by heating with potassium hydroxide might be ascribed to methionine. Moreover, the same author has reported that *l*-methionine gave methyl mercaptan by the action of microorganisms, but not hydrogen sulphide even by the same microorganisms.

In the literature, the report on the electrolytic oxidation of methionine could not be found. The present authors (1938) obtained hydrogen sulphide, methyl mercaptan and β-methylsulphone-propionic acid, and repotred these facts at the annual meeting of the Chemical Society of Japan in 1939. At that time, the authors had isolated methionine-sulphone, but gave only notice of its presence, as the analysis was not completed. Afterwards it was confirmed by the authors that the methionine-sulphone thus obtained was in its optically active form: $[\alpha]_D^{19^\circ}=+11.50$.

Recently, Virtanen, Laine, and Toivonen (1940)(5) intended to esti-

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Translated by the authors from *J. Chem. Soc. Japan*, **63** (1942), 485.

(1) Y. Takayama, T. Harada and S. Miduno, this Bulletin, **12** (1937), 342.

(2) L. W. Butz and V. d. Vigneud, *J. Biol, Chem.*, **99** (1932), 135; L. W. Butz, *ibid.*, **97** (1932), xxi.

(3) G. Toennies, J.J. Kolb· *J. Biol. Chem.*, **128** (1938), 399; G. Toennies, *Science*, **88** (1938), 545, *Chem. Abstracts*, **33** (1939), 1271.

(4) J. *Onitake, J. of the Osaka Medical Soc* , **37** (1938), 263, Onitake and I hihara, *ibid.*, **37** (1938), 561.

(5) A I Virtanen, T Laine and T. Toivonen *Z. Physiol. Chem.*, **266** (1940), 193.

mate methionine as the dimedon compound of methylthiopropionaldehyde formed by its oxidation with ninhydrin. More recently Toennies (1941)⁽⁶⁾ has obtained *dl*-methionine-sulphone from methionine by its oxidation with hydrogen peroxide in the presence of molybdate and perchloric acid. Tsuchiya (1941)⁽⁷⁾ has reported on the detection of methionine by the colour reaction of methyl mercaptan formed by the action of alkali on it.

The authors electrolysed *l*-methionine prepared⁽⁸⁾ from the leucine-fraction of the hydrolysate of soy bean protein, in dilute sulphuric acid at 100° or at ordinary temperature.

In the case of 100°, the electrolysis was tried in an undivided cell with a lead peroxide anode and a lead cathode. During the electrolysis, the volatile oxidation products from the cell was distilled through the delivery tube into a receiver, as described in Part XI. The volatile oxidation products containing sulphur were confirmed as hydrogen sulphide and methyl mercaptan. As non-volatile oxidation products, β-methylsulphone-propionic acid and *l*-methionine-sulphone were isolated.

In the case of ordinary temperature, the electrolysis was tried in a divided cell under the following conditions.

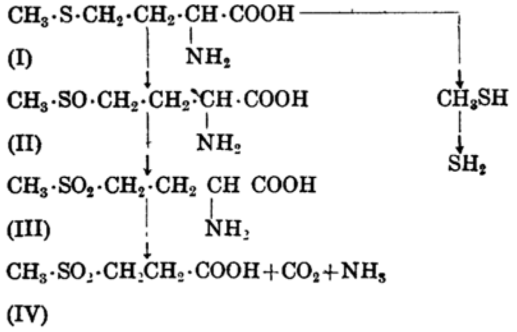
Electrodes: Lead peroxide anode and lead cathode; Cell: an inner porous pot served as the anode compartment and an outer glass cylinder as the cathode compartment.

In the first case: Anolyte, solution of methionine in dilute sulphuric acid; catholyte, dilute sulphuric acid.

In the second case: Anolyte, dilute sulphuric acid; catholyte, solution of methionine in dilute sulphuric acid.

In the first case, the intensity of colour reaction of methyl mercaptan formed in the anode compartment gradually increased, while the colour reaction of the methyl mercaptan produced in the cathode compartment was slower in its appearance, but higher in its intensity than that of the gas formed in the anode compartment, as the electrolysis proceeded.

In the second case, hydrogen sulphide and methyl mercaptan were not detected in the anode gas. In the cathode gas, hydrogen sulphide was detected when the current quantity amounted 2F/mol, and its formation



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(6) G. Toennies and J. Kolb, *J. Biol. Chem.*, 140 (1941), 131.

(7) Y. Tsuchiya, *Bulletin of the Agricul. Chem. of Japan*, 17 (1941), 45.

(8) Y. Takayama and Y. Tsuchiya, *ibid.*, 17 (1941), 61.

seemed to be favoured by heating, but methyl mercaptan was not detected.

From these facts, it may be concluded that in the formation of methyl mercaptan the anodic oxidation of methionine seems to be necessary, and the formation of hydrogen sulphide may be ascribed to the reduction of methionine and methyl mercaptan. In fact, the formation of hydrogen sulphide from methyl mercaptan by cathodic reduction was confirmed experimentally, as is described below.

From the above results, the mechanism of the electrolytic reaction of methionine in dilute sulphuric acid may be represented as follows:

l(−)Methionine(I) is first oxidised to *l*(+)methionine-sulphoxide (II) which is rapidly oxidised to *l*(+)methionine-sulphone(III), then *l*(+)methionine-sulphone is oxidised to methylsulphone-propionic acid (VI) with the splitting-off of ammonia and carbon dioxide. The mechanism of the formation of methyl mercaptan can not be concretely elucidated, but it seems probable that methionine is first oxidised and then produces methyl mercaptan. It may be probably said that hydrogen sulphide is the cathodic reduction product of methionine or methyl mercaptan. In these experiments, it was found that the sulphur atom of methionine was more sensitive than its amino-group to anodic oxidation.

One of the authors (Miduno)⁽⁹⁾ has further studied the mechanism of the oxidation of methionine and reported the results in the Journal of the Chemical Society of Japan (63(1942), 1317).

Toennies and Kolb, as described above, obtained methionine-sulphone from methionine by oxidation with hydrogen peroxide only when two catalysers were present, while the present authors easily obtained it in its optically active form by electrolysis under suitable conditions, moreover in good yield.

Methyl-mercapto-propioaldehyde and its alcohol which are the derivatives of methionine are some components of the essence of soy. Some part of the smell produced from protein by hydrolysis with strong acid will probably be due to the oxidation and reduction products of methionine.

Experimental.

1. The gaseous substances and organic acids containing sulphur produced from crude leucine.

A. *The gaseous substances containing sulphur.* It was found that the gaseous mixture with disagreeable smell evolved during the electrolysis of crude leucine at 100° contained hydrogen sulphide and methyl mercaptan. Hydrogen sulphide was fixed by dry lead acetate. Methyl mercaptan was precipitated with mercuric cyanide. The precipitate was washed twice with alcohol and then with water. It was dried in a desiccator and its sulphur was determined by Bailey's method. (Found: S, 21.67;

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(9) The oxidative decomposition of *l*-methionine-sulphoxide and *l*-methionine-sulphone by electrolysis.

21.65. Calculated for $(CH_3S)_2Hg$: S, 21.72%).

B. *Non-volatile acid containing sulphur.* The electrolysate in the cell was neutralised with sodium hydroxide and concentrated. The crystals (sodium sulphate), which separated out, were filtered off. The filtrate was acidified with sulphuric acid and distilled with steam to separate the volatile acids. The remaining solution was extracted with ether. From the ethereal solution, crystalline substance was separated and identified as succinic acid: m.p. 184–185°. (Found: N/10-NaOH, 17.75 cc. Calculated for $C_4H_6O_4$: N/10-NaOH, 17.91 cc. Found: Ag, 64.35. Calculated for $C_4H_4O_4Ag_2$: Ag, 64.63%).

The mother liquor of succinic acid was neutralised with barium hydroxide and concentrated. The separated barium succinate was filtered off. The filtrate was treaetd with sulphuric acid, and the liberated acid was neutralised with sodium hydroxide and evaporated up. The mass thus obtained was treated with concentrated hydrochloric acid and evaporated to expel the excess of hydrochloric acid, and then extracted with alcohol (99%). After removal of alcohol, crystals were obtained and recrystallised from water (m.p. 102–104°) and then from alcohol. It was identified as β-methylsulphone-propionic acid, $CH_3SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$. This acid coincided with the acid which was obtained by Schneider (1910)⁽¹⁰⁾, and Barger and Coyne (1928)⁽¹¹⁾. The yield was 17 g. from 1 kg. of crude leucine. (Found: N/10-NaOH, 13.13 cc; S, 21.51. Calculated for $C_4H_8O_4S$: N/10-NaOH, 13.15 cc; S, 21.08%).

II. Electrolysis of methionine at 100°.

The electrolysis of methionine was undertaken in the same apparatus as described in Part XI. The volatile substances distilled with steam were conducted into a receiver, and the uncondensed gas was led into an ice-cold washing bottle containing water and then dried with calcium chloride. Hydrogen sulphide in the gas thus treated was fixed with solid lead acetate by Reith's method and the methyl mercaptan in the gas was caught in an isatin-sulphuric acid or precipitated as mercaptide with mercuric cyanide. The electrolysate in the cell was treated with ether. From the part soluble in ether, β-methylsulphone-propionic acid was isolated, and from the insoluble part ammonia and *l*-methionine-sulphone were isolated.

The above-mentioned electrolysis was carried out under the following conditions.

Electrolysate: *l*-Methionine (1.4980 g, 10 m. mol) dissolved in N-H_2SO_4 (32 cc). Electrodes: Anode PbO_2, 2×5 cm. Cathode Pb, 2×5 cm. C.D.: 2 amp/dm². 2.7 volts, 8F/mol, 100°, NH_2-N/T.N., 77.3% (after electrolysis).

A. *Volatile substances. The confirmation of hydrogen sulphide and methyl mercaptan.*

Hydrogen sulphide was fixed on dry granular lead acetate in absorp-

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(10) W. Schneider, *Ann* 375 (1910), 234.

(11) G. Barger and F. P. Coyne, *Biochem. J.*, 22 (1928), 1417.

tion tubes, and methyl mercaptan was precipitated with mercuric cyanide. On decomposing the precipitate with dilute hydrochloric acid, free methyl mercaptan was obtained, and tested with sodium nitroprusside, isatin-sulphuric acid (red), mercuric chloride (milky white precipitate) and lead acetate (yellow precipitate). (Found: S, 20.30. Calculated for $(CH_3S)_2Hg$: S, 21.72%).

Neutral and acidic substances distilled with steam. Acidic part in the distillate (102 cc) required 47.1 cc of N/10-NaOH. Neutral part in the distillate gave the positive reaction of fuchsin-sulphurous acid solution, and gave a little precipitate of *p*-nitrophenylhydrazone.

B. *Non-volatile substances with steam. β-Methylsulphone-propionic acid.*

The electrolysate was concentrated and extracted with ether. On expelling ether from the ethereal solution, a syrupy solution was obtained. After neutralising the solution with barium hydroxide, it was concentrated and precipitated with absolute alcohol. The syrup with precipitates was dissolved in water and freed from $Ba^{\cdot\cdot}$ and SO_4'' as usual, then decolourised, and concentrated to crystalise. The crystals (1.3 g) were recrystalised from water: m.p. 100–103°. It was identified as β-methylsulphone-propionic acid (Found: N/10-NaOH, 10.34 cc; S, 21.12. Calculated for $C_4H_8O_4S$: N/10-NaOH, 10.31 cc; S, 21.08%).

Ammonia and *l*-methionine-sulphone. The above-described residue of ethereal extraction was alkalinised with an excess of barium hydroxide and distilled at 60–70° under reduced pressure. The distillate was caught in hydrochloric acid (hydrochloride 2.313 g). It was identified as ammonia. (Found: Pt, 43.66. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

The solution freed from ammonia was saturated with carbon dioxide to precipitate $Ba^{\cdot\cdot}$ and concentrated to crystalise. The crystals (2.31 g) were separated, and from the mother liquor more crystals (0.718 g) were obtained on adding absolute alcohol. They were recrystalised from water (m.p. 245–246°) and confirmed as *l*-methionine-sulphone. (Found: N, 7.74; NH_2-N, 7.69; S, 18.33. Calculated for $CH_3SO_2C_4H_8O_2N$: N, 7.73; NH_2-N, 7.73; S, 17.69%). Copper salt (Found: N, 6.61. Calculated for $CH_3SO_2C_4H_7O_2N\ Cu/2$: N, 6.53). $[\alpha]_D^{19°}=+11.5°$.*

III. Electrolysis of methionine at ordinary temperature (about 20°).

The electrolysis of methionine was undertaken in an anode solution as well as a cathode solution at ordinary temperature and its details were as follows:

Apparatus and treatments. A cylindrical lead peroxide anode (5×8 cm.) was placed in a porous pot which served as the anode compartment. A cylindrical lead cathode (6×13.5 cm.) was placed in an outer glass jar and encircled the porous pot. The inner porous pot was provided with a rubber stopper carrying two glass tubes, the one being an air inlet tube, and the other a gas delivery tube. The outer jar had a rubber ring

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* *l*(+)Methionine sulphoxide $[\alpha]_D^{19°}=+5.4°$.

stopper which was also provided with similar two tubes.

To separate the volatile substances such as hydrogen sulphide and methyl mercaptan evolved from the electrolysate, a current of air was passed during the electrolysis under the same conditions, through both the anode and the cathode compartments.

The gaseous substances together with the air were passed through an ice-cold washing bottle containing water, and led into tubes with dried granular lead acetate to fix hydrogen sulphide, and then into an isatin-sulphuric acid solution or a mercuric cyanide solution (3%) to catch methyl mercaptan. Finally, the gas was collected over water.

A. *The electrolysis of methionine in the anode solution.*

A solution of methionine (1.492 g, 10 m. mol) in N-H_2SO_4 (34 c.c.) was electrolysed in the anode compartment, the catholyte being N-sulphuric acid (70 c.c.). Anodic current density: 2 amp./dm.^{2}, 3 volts, 20°.

Methyl mercaptan. From the beginning of the electrolysis, the anode gas changed the colour of the isatin-sulphuric acid gradually to green. and when the current quantity amounted to 3F/mol, its characteristic green colour developed, while the cathode gas began to change the colour of the solution from 4F/mol and finally gave more intense green colour than that given by the anode gas.

After the current quantity amounted to 12F/mol, both gases were absorbed, separately in the mercuric cyanide solution (3%), more current 2F/mol being passed. The precipitates of methyl-mercaptide given by both gases were far less than in the electrolysis at 100°.

Hydrogen sulphide. The anode gas gave no reaction of hydrogen sulphide, while the cathode gas began to form lead sulphide from 4F/mol and yielded the layer of 2 mm. of lead sulphide.

B. *The electrolysis of methionine in the cathode solution.*

A solution of methionine (1.4923 g, 10 m. mol) in N-H_2SO_4 (65 c.c.) was electrolysed in the cathode compartment, its current density being 1 amp./dm.^{2}, and the anolyte N-H_2SO_4 (35 c.c.). All the treatments were same as in the case of the electrolysis in the anode compartment. The anode gas contained neither hydrogen sulphide nor methyl mercaptan. In the cathode gas, hydrogen sulphide was detected from 2F/mol and its amount appeared to increase by heating, but methyl mercaptan was not detected.

IV. The electrolysis of methyl mercaptan at ordinary temperature.

Methyl mercaptan free from hydrogen sulphide was electrolysed in the above-described cell. When the electrolysis of methyl mercaptan was undertaken in the cathode compartment (C.D.: 1 amp./dh.^{2}), hydrogen sulphide was confirmed with both lead acetate paper and $CdCl_2$-solution. On the contrary, in the case of the anode compartment no trace of hydrogen sulphide was detected.

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