LXXXIX. SOME CHEMICAL CONSTITUENTS OF THE MUSSEL (*MYTILUS EDULIS*).

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INTRODUCTORY.

TISSUES of the common sea-mussel (*Mytilus edulis*), macerated in 60 % alcohol, during another investigation gave an extract which, after filtering and further addition of alcohol, deposited clusters of colourless crystals on standing. Experiment showed that this crystalline substance was much more soluble in water than in alcohol and could always be thrown out by further addition of alcohol to the tissue-extracts. Within a range of 70–80 % alcohol, two distinct crystalline forms were observed together with a certain amount of brownish coloured gummy material. At alcoholic strengths nearer to 70 % the predominant form of the crystals was that of prismatic needles. From higher concentrations of alcohol the crystalline deposit was chiefly in the nature of stout hexagonal plates.

Previous work with the mussel had been focussed principally upon the possible causes of poisoning in human beings after eating the mollusc [Fürth, 1903] and Brieger [1889] isolated a supposed specifically poisonous body which he called mytilotoxin, the existence of which has been disputed by Thesen [1902].

Kelly [1904], from analysis of the sulphur content of muscular tissue of *Mytilus*, calculated that this should contain 5 % of taurine and that the Bojanus organs should yield 1.95 % of that substance. By extraction of these tissues with boiling water, precipitation of the extract with lead acetate solution, and evaporation of the filtrate (freed from lead by H_2S treatment), followed by acidification, taurine was isolated, but the yields in both experiments were much lower than was expected.

During the course of a comparative study of different kinds of muscle, Jansen [1917] made a chemical examination of the extract of the posterior adductor muscle of *Mytilus edulis*. Following the procedure of Kutscher [1905] he isolated betaine, taurine (0.08 % on wet flesh) and a new substance of characteristic properties, from the analysis of which he deduced the formula $\text{C}_6\text{H}_{12}\text{O}_5 \cdot 2\text{H}_2\text{O}$ corresponding to a *cyclo*-hexanepentitol

isomeric with quercitol and *iso*-quercitol, which he called mytilitol.

By the action of acetic anhydride upon the compound, Jansen obtained an acetyl derivative and found microscopical needle crystals melting at 182° which apparently contained five acetyl groups.

In 1921 a new chemical examination of the aqueous extract of *Mytilus* was undertaken by Ackermann [1921, 1], who stated that he was unaware of Jansen's discovery of mytilitol until he had completed his experiments, which demonstrated the existence of a ring-alcohol in the tissues of the animal. The physical and chemical constants of the new substance, also called mytilitol, show considerable divergence from those given by Jansen. In particular, the melting point of mytilitol according to Ackermann is 11° lower than that given by the earlier investigator. The percentages of carbon and hydrogen also differ to some extent and from these differences, together with a marked discrepancy in the proportion of water of crystallisation in the molecule compared with that theoretically required for $C_6H_{12}O_5 \cdot 2H_2O$, Ackermann assigned a new formula for mytilitol which he designated a methyl-cyclo-hexanehexitol, $C_7H_{14}O_6 \cdot 2H_2O$.

Since preliminary chemical tests showed the hexagonal plates obtained in the present experiments to have similar properties to those of Jansen's mytilitol, a critical examination of the substance was undertaken with the object of deciding if possible between the two formulae.

EXPERIMENTAL.

(i) *Extraction of mussel powder.* Material was available in the form of dry powder which had been obtained by removing the mussels from their shells and drying the comminuted flesh at 105° , after which the dried mass was ground in a handmill. The fat was extracted with light petroleum in a Soxhlet apparatus and the friable powder after this treatment was soaked in its own bulk of water and allowed to stand for 48 hours. The clear extract was taken down *in vacuo* to small bulk. Colloidal ferric hydroxide solution (Liq. Ferri Dialysati, B.P.) was then added until there was no further precipitation. In order to coagulate the excess of colloidal ferric hydroxide it was found necessary to boil the liquid with animal charcoal. After filtration absolute alcohol was added until the strength of the mixture was 80 %, whereupon a flocculent precipitate, resembling glycogen, was brought down. From the filtrate after standing a good crop of hexagonal plates was obtained.

Further tests showed that this cold water extraction was not complete.

(ii) *Extraction of the wet mussel flesh.* The use of 60 % alcohol as an extraction fluid avoids a good deal of contamination with glycogen in subsequent operations. The original extractions involve comparatively large volumes of liquid, since the wet flesh of *Mytilus* contains as a rule about 80 % of water. The calculated amount of strong alcohol must therefore be added to obtain the required final strength of 60 %. The live mussels after shelling were put through a mincing machine and the necessary amount of alcohol

added. After thorough shaking the mass was allowed to stand 1 or 2 days in an ice-chest.

The clear extract was decanted and the residual sludge squeezed in bags of miller's gauze of fine mesh; the expressed liquid was then filtered and added to the clear extract.

The combined liquors were reduced considerably in bulk and filtered, after which the suspended fatty material was removed by ether extraction. This operation proved troublesome owing to the formation of emulsions. Strong alcohol was now added and a flocculent precipitate filtered off, but some brown oily material passed through the filter. In order to eliminate this the liquid was boiled with animal charcoal and refiltered.

The clear liquid was then made up with strong alcohol to 80 % strength, since that was the concentration at which crystalline deposits were first observed.

On standing in the ice-chest crystals of two distinct types were thrown down, together with a small amount of brown sludge¹. Some hexagonal nodules or stout plates and a larger proportion of prismatic needles were thus isolated and from the mixture the needles could be partially separated by hand.

The crystals remaining after this separation were dissolved in the minimum amount of water and the solution, after boiling with animal charcoal, was filtered. Upon adding strong alcohol until the concentration reached 75–80 % and then allowing the solution to stand, the hexagonal plates were obtained in a pure state. Chemical examination, the results of which are appended, showed the hexagonal plates to be a body similar to Jansen's mytilitol. The needle prisms proved to be taurine.

Using the methods described above, starting from either dry powder or wet tissue, it is not claimed that the yields are quantitative. In no case was the mother-liquor completely exhausted, as evidence of further slight deposit was always obtained on continued increase of alcoholic concentration.

The scheme for quantitative determination of inositol recently worked out by Needham [1923] was not applicable for the following reasons. Needham's process consists of a micro-carbon estimation on the pure cyclose precipitated by alcohol from aqueous solutions after successive treatments with neutral and basic lead acetate. In the case of mytilitol, difficulties of separation were experienced in several preliminary attempts to utilise the lead acetate method. It may be noted in this connection that H. Müller [1907] found that cocosite, isomeric with inositol, actually entered into chemical combination with the lead acetate used in his experiments.

The following figures calculated on the wet tissue of *Mytilus* indicate the range of yields obtained from animals just about to spawn:

Exp. 1. Crop of pure needle prisms weighed 1.19 g. obtained from 1910 g. wet flesh = 0.062 %.

¹ This latter material contained nitrogenous compounds and yielded methylamines on warming with alkali [cf. Ackermann, 1921, 2].

Exp. 2. Crop of pure needle prisms weighed 0.21 g. obtained from 796 g. wet flesh = 0.026 %.

Exp. 3. Crop of pure hexagonal plates weighed 0.458 g. obtained from 1951 g. wet flesh = 0.024 %.

The deposition of either or both of the crystalline bodies from the different extracts was found to be a matter of some variation. This behaviour was probably governed by the varying proportions of each substance present in the several experiments and by their respective solubilities, which are given in the literature as follows [cf. Jansen, 1917; Ackermann, 1921, 1]:

Mytilitol, 0.43 % soluble in water at 24°. Insoluble in absolute alcohol.

Taurine, 6.25 % soluble in water at ordinary temperatures. Insoluble in absolute alcohol.

(iii) *Chemical examination of the crystals.* The prismatic needles on careful heating melted with decomposition at approximately 300°; and, in other respects, had the properties of taurine, $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SO}_3\text{H}$. This was confirmed by micro-analysis.

The *hexagonal plates* contained only carbon, hydrogen and oxygen and were free from ash; M.P., 272° (uncorr.). The crystals were slightly soluble in water and insoluble in absolute alcohol; the aqueous solution was optically inactive. Scherer's reaction always gave negative results, although different modifications of the conditions were tried. It may be noted, however, that Jansen records a positive Scherer test with mytilitol, and Ackermann describes the reaction as weakly positive. The following results were obtained on analysis:

Micro-analysis found: 15.8 H₂O %, 42.99 C %, 7.72 H %.

Macro-analysis found: 15.71 H₂O %, 43.16 C %, 7.37 H %.

Calculated for $\text{C}_7\text{H}_{14}\text{O}_6 \cdot 2\text{H}_2\text{O}$: 15.66 H₂O %, 43.28 C %, 7.27 H %.

Acetylation. The substance, when heated upon the water-bath for 1 hour with acetic anhydride to which a trace of concentrated H₂SO₄ had been added, completely dissolved. After cooling, the unchanged acetic anhydride was decomposed by the addition of hot water and the solution on standing deposited colourless prismatic needles. These, after separation and drying, were recrystallised from absolute alcohol or from glacial acetic acid, and melted sharply at 182° (uncorr.), which figure agrees with that given by Ackermann for hexacetylmethyl-*cyclo*-hexanehexitol. Jansen, on the other hand, designates the body melting at this temperature a pentacetate. Both authors rely upon quantitative determination of the acetyl groups by saponification for confirmation of their respective formulae.

CONSTITUTION OF THE ACETYL COMPOUND.

From Jansen's results, stated in column (iv) of Table I, it will be seen that he might have chosen either formula for the fully acetylated body (see columns (i) and (iii)).

From mytilitol by partial acetylation Ackermann prepared a compound melting at 159° which contained 52.59 % of CH₃.CO.

The percentages calculated for the three compounds formulated in Table I are (i) 57.5, (ii) 53.4 and (iii) 57.85.

Table I. *Mg. acetic acid by titration after saponification.*

	(i)	(ii)	(iii)	(iv)
	Calculated for $C_6H_7(O.CO.CH_3)_5$ fully acetylated pentitol	Calculated for $C_7H_8(OH)(O.CO.CH_3)_5$ pentacetyl hexitol	Calculated for $C_7H_8(O.CO.CH_3)_6$ fully acetylated hexitol	Found by Jansen
Exp. 1	278.6	257.9	280.4	271.2
Exp. 2	186.9	173.0	188.1	190.2

By complete acetylation of mytilitol, Ackermann obtained a substance melting at 182° , and since he had already isolated the pentacetyl derivative (M.P. 159°) he concluded that the higher melting body was hexacetylmytilitol.

Analysis for carbon and hydrogen does not assist in making a choice between the various possible acetyl derivatives, since the percentage differences are of the order of experimental error.

CONSTITUTION OF MYTILITOL.

In support of his formula Jansen also determines the percentages of carbon and hydrogen on the hydrated form of the original compound, but such analysis fails to decide between the two possibilities as shown:

Calculated for $C_6H_{12}O_5 + 2H_2O$: 35.98 C %, 8.06 H %.

„ $C_7H_{14}O_6 + 2H_2O$: 36.50 „ 7.88 „

Jansen found: 36.30 „ 8.10 „

The critical figure, however, which enables a decision to be made, is the percentage of water of crystallisation, and this was left undetermined by Jansen.

The results of the present investigation confirm those of Ackermann whose formula for mytilitol as a methyl-*cyclo*-hexanehexitol

is therefore, in our opinion, the correct one.

The appended tables summarise the data upon which this conclusion is founded.

Table II.

	% C	% H	Hydrated compound % H_2O of crystallisation
Calculated for $C_6H_{12}O_5$ anhydrous	43.89	7.37	18.01
„ „ $C_7H_{14}O_6$ „	43.29	7.27	15.65
Found by Jansen	43.7	6.9	—
„ „ Ackermann (a) macro-analysis	43.05	7.33	15.93
„ „ „ (b) micro-analysis	43.40	7.08	—
„ „ „ (c) „	43.44	7.21	15.83
„ „ „ (d) „	43.16	7.27	15.62
„ in present investigation (i) micro-analysis	42.99	7.72	15.80
„ „ „ (ii) macro-analysis	43.16	7.37	15.71

Table III.

	M.P. of mytilitol	M.P. of acetyl derivative
Jansen	270°	182° (completely acetylated)
Ackermann	259°	(i) 157-8° (pentacetyl) (ii) 180-1° (hexacetyl)
Present investigation	273°	(a) 182° (b) 182.5°

(a) Using acetic anhydride containing trace of concentrated H_2SO_4 .

(b) Obtained by action of *pure* acetic anhydride in attempting to prepare the pentacetyl compound.

CHEMICAL SIGNIFICANCE OF MYTILITOL AND TAURINE.

Jansen concluded from his experiments that mytilitol was localised in the posterior adductor muscle, which is well supplied with nerve fibres and is capable of exerting considerable force in keeping the shell-valves tightly closed. As this is the sole function of the muscle, one is led to assume that mytilitol has some vital connection with the supply of energy necessary for such work.

Since the consensus of opinion is in favour of muscular work being performed at the expense of carbohydrates [Leathes and Raper, 1925], a highly oxygenated body of similar type such as mytilitol would appear to be a material which, on combustion, might yield the energy required by the muscle in activity.

In the case of vertebrates, lactic acid is regarded as an end-product of chemical change in the muscles, and according to Embden [1921] it is developed from a parent substance, "lactacidogen," which is a hexosediphosphoric acid derived from glycogen.

Although Jansen did not find lactic acid in the adductor muscle of *Mytilus*, the possible existence of a "lactacidogen" is not excluded, since during the resting stage of muscle the acid may disappear, partly as CO_2 , and partly by being converted back into glycogen [Meyerhof, 1923].

The widespread occurrence of inositols combined with phosphoric acid, as in phytin, suggests analogy with mytilitol in this connection. Quercitol yields malonic acid by the action of KMnO_4 and therefore the oxidation of mytilitol might give a mixture of tartronic (hydroxymalonic) acid, $\text{CH}(\text{OH})(\text{COOH})_2$, and methyltartronic acid. The latter substance, $\text{CH}_3.\text{C}(\text{OH})(\text{COOH})_2$, is known to lose CO_2 readily with formation of lactic acid and, by oxidation, yields pyruvic acid.

In any case, both tartronic acids would be easily oxidised to CO_2 *in vivo* [Dakin, 1922].

In the presence of water and under anaerobic conditions the oxygen required for the breaking down of mytilitol *in vivo* might be supplied in either or both of two ways. Firstly, there is the possible agency of soluble oxidases of the type already proved to occur in tissues capable of oxidising citric, tartronic, lactic, pyruvic acids and other substances [Thunberg, 1920; Stern, 1914].

Secondly, there is the glutathione oxidation-reduction system which has been found by Hopkins [1921] in many kinds of tissue, including that of the common oyster. The known relationship between taurine, cystine, and cysteine suggested the possibility of *Mytilus* containing a system of similar type.

Qualitative experiments were therefore carried out as follows. (a) The comminuted whole tissue of several mussels was treated with a few drops of acetic acid and then ammonium sulphate was added to saturation; the mixture after standing overnight was filtered through calico on a Buchner funnel. The clear extract gave a deep magenta colour upon the addition of a 5 % solution of sodium nitroprusside, followed by ammonia in excess. (b) Live mussels were dissected and the fresh tissues tested, after suspension for a short time in saturated ammonium sulphate solution, by adding the nitroprusside reagent and finally a few drops of ammonia. The adductor muscle gave a positive reaction which did not rapidly fade. The "foot" muscle gave a weakly positive reaction. The gastric gland gave uncertain results, the colour reaction being masked by the natural pigment of the organ. The mantle containing female reproductive products gave a strongly positive reaction which faded rapidly.

It is possible that taurine, which is also found in *Mytilus*, results from the hydrolysis of glutathione, followed by oxidation of the cysteine set free, since Friedmann [1903] has shown that the latter may be oxidised to cysteic acid, $\text{CH}_2(\text{SO}_3\text{H})\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$, which yields taurine by loss of CO_2 .

Possibly also the parent substance cysteine may be converted partly into the oxidised form of glutathione and partly into taurine according to the scheme:

Hopkins [1921] has pointed out that the nitroprusside reaction is much less intense in cold-blooded animals than in warm. He suggests that this may be a matter of tissue equilibrium favouring the oxidised form of glutathione.

This substance can act as a "hydrogen acceptor" under certain conditions and might therefore function in the oxidation of mytilitol according to the mode outlined above.

Taurine is found especially abundant in the muscles of cold-blooded animals [Hammarsten, 1922], and this fact strongly supports the idea of a connection with oxidised glutathione.

With regard to the reserve-products of the mussel, a large proportion of glycogen is always present in the connective tissue and evidence is not wanting

that glycogen is used in production of fat during the extraordinary activity of the reproductive period [Daniel, 1921].

Whilst it is difficult to see how mytilitol could be derived from either of these reserve substances, it is nevertheless suggestive that when mussel-flesh is dried there is a considerable divergence between the amounts of glycogen in the fresh material and the quantities estimated after drying the tissue, and this loss is not accompanied by any evidence of the formation of dextrin or reducing sugars.

Examination of *Ostrea edulis* has afforded similar results, the disappearance of glycogen during the drying of oyster-meat in some cases corresponding to more than half the amount of glycogen originally present, and here also there were no indications of the formation of a reducing substance [Russell, 1923]. From this point of view therefore it would be interesting to know whether a mytilitol-like body is present also in the oyster.

NOTE ON THE FATTY MATTER PRESENT IN *MYTILUS EDULIS*.

During the experiments on the isolation of mytilitol difficulties were encountered owing to the presence of fatty oil in the alcohol liquors. The character of this fatty matter suggested high unsaturation and, with the object of gaining further information about the oil in a condition as far as possible unaltered, an extraction from fresh material was undertaken¹.

The minced flesh of fresh mussels was mixed with the calculated amount of anhydrous sodium sulphate together with sufficient plaster of Paris to ensure a friable mass. This was percolated with repeated quantities of light petroleum (boiling below 40°).

After evaporation of the solvent in a stream of CO₂ the residual viscid oil was dark green in colour with a faint fluorescence, and on standing showed a tendency to deposit waxy crystalline matter, at the same time readily forming an elastic skin, even when stored in an oxygen-free atmosphere. Notwithstanding the strong evidence of the presence of lecithin-like bodies, these were not investigated because of the small quantity of oil available.

The following analytical figures were obtained by examination of the material as soon as possible after extraction:

Sp. gr.: 0.972 at 15.5°.

Ref. ind.: 1.495 at 15.5°.

Iodine value: 140–150 (range of several determinations by the standard method of Wijs).

Unsaponifiable matter: 6.0 % (which gave positive reactions for cholesterol when tested by methods of Salkowski-Hesse [1872, 1878], Lifschütz [1908] and Whitby [1923], and contained 36.1 % of its weight of cholesterol estimated by the digitonin method of Windaus [1910]).

Cholesterol (calculated on the original oil): 2.15 %.

¹ The fat in the wet flesh of *Mytilus* ranges from 2.0 % immediately before spawning-time in the spring to 0.5 % after spawning, and therefore really demands extraction on the large scale.

The iodine number indicates a fair degree of unsaturation of the oil and suggests the presence of highly unsaturated fatty acids. Working with larger quantities, it should be possible to effect a separation of the lipins from the true fats, and by further determinations to verify this conclusion.

SUMMARY.

1. The extraction by aqueous alcohol of taurine and mytilitol from the common sea-mussel is described.
2. Experimental evidence is produced in confirmation of the methyl-*cyclo*-hexanehexitol structure given to mytilitol by Ackermann, as opposed to Jansen's *cyclo*-pentitol formula.
3. Qualitative tests indicate the presence of a glutathione-like body in the tissues of *Mytilus*.
4. The chemical functions of mytilitol, taurine and glutathione are discussed.
5. A preliminary examination of the fatty matter of *Mytilus edulis* shows it to contain cholesterol (2.15 %) and probably highly unsaturated fatty acids.

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