XXXV.—*Researches on Leucimide, a Decomposition product of the Albuminous Substances and of Leucine.*

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THE products of the decomposition of albuminous substances by the usual process of Braconnot, before any leucine or tyrosine has been abstracted therefrom, are cautiously evaporated to dryness, and further dried by prolonged exposure to air. The residue is now stirred in a mortar with absolute ether, and then digested with the ether for several weeks in a bottle, and frequently shaken. The decanted solution, after distillation of the ether, leaves white crystals, which are further purified from an adhering syrup by washing with water and recrystallisation from boiling absolute alcohol.

Instead of the ether, alcohol may also be employed for the first extraction of the white crystals from the dried mixture. A dark alcoholic solution is obtained, which, after evaporation at a low temperature, on cooling deposits crystals in broom-like masses. These crystals are insoluble in water, and from this mother-liquor a considerable quantity of the matter composing these crystals is precipitated by the addition of water. After

washing on the filter, these flakes and crystals are easily soluble in boiling alcohol. The brown solution is decolorized by treatment with animal charcoal, and after concentration and standing, deposits colourless crystals in voluminous delicate masses. After drying they had the properties and composition of leucimide, hereafter described.

In several most laborious operations with large quantities of material (in one instance as much as fifty pounds of animal hair having been operated upon by seventy pounds of sulphuric acid, and the product after evaporation in a vacuum apparatus having been extracted by many gallons of ether and alcohol), only a few grammes of substance were obtained. These only just sufficed for establishing the identity of the substance with leucimide, whereupon the study of the properties of this body was continued upon material obtained by dehydration of leucine.

Leucine obtained from animal tissues by sulphuric acid and purified, was placed in a Florence flask and heated in an oil-bath, while a current of dry hydrochloric acid gas was passed through it. At the temperature of 210° — 215° C., the crystals of leucine disappeared, and a brownish semi-fluid mass collected at the bottom of the flask. During the whole time the current of acid carried a quantity of water-vapour into the discharge tube and condensing flask attached, as also some oily drops. When the temperature of the oil reached 230° C., a copious white sublimate appeared in the neck of the flask. The action was then interrupted, and the product purified by extraction with water and repeated crystallisation from alcohol. By repeating the operation I have learned that it is advantageous to continue the heat to about 230° until the sublimate appears, as otherwise more leucine remains in the state of hydrochlorate; the resinous product can be treated with hot water directly, but it is easier to dissolve it first in a small quantity of alcohol and to pour this into a large quantity of water. The washed precipitate crystallises easily and pure from alcohol, while the water retains all other admixtures.

The products soluble in ether, can be obtained in the following manner and succession:—

Oil.—On gentle evaporation a yellowish brown oil is deposited in films and drops. It is easily soluble in ether, partially soluble in caustic potash. It is also found in the volatile

products, together with hydrochlorate of amylamine. The oil is insoluble in water, but very easily soluble with a yellow colour in free amylamine.

Amylamine.—From the concentrated water extract, and from the volatile oil this base is obtained by distillation with caustic potash. It is crystallised with hydrochloric acid, and purified by redistillation. The solution in water of the dry hydrochlorate is precipitated by bichloride of platinum, and some alcohol. The resulting light yellow compound left platinum.

Leucine-hydrochlorate.—The caustic mixture on cooling still deposits some leucimide, after removal of which a body crystallises in white hard granules. It is leucine-hydrochlorate of potassium, very little soluble in caustic-potash mixture, more easily soluble in, and not recrystallised from pure water.

Ammonia.—A vestige of this base is also sometimes obtained. If the oil be considered as the second leucic acid anhydride, the ammonia may be claimed as the product of decomposition of leucimide.

Physical and Chemical Properties of Leucimide.—The substance howsoever obtained crystallises in needles of the rhombic system, visible under high powers of the microscope. They are white, and of a dull, glistening, cotton-like appearance. When heated in a test-tube they fuse, and then sublime easily, the vapours condensing in voluminous clouds of most delicate needles.

They are insoluble in cold water, but a little soluble in boiling water, the solution becoming turbid on cooling. They are easily soluble in cold alcohol, more so in hot alcohol; a boiling saturated solution on cooling solidifies into a mass of crystals. The addition of large quantities of water to an alcoholic solution precipitates the leucimide almost completely in flakes. In ether leucimide is less soluble than in alcohol.

The crystals of leucimide are not changed by caustic ammonia or by concentrated caustic potash even on boiling. Cold concentrated hydrochloric acid also leaves them unchanged; on boiling a trace dissolves, which makes the solution turbid on cooling. Concentrated acetic acid keeps leucimide in solution, and yields it as a copious precipitate on the addition of alkali. Concentrated nitric acid also dissolves leucimide and leaves it unchanged even on evaporation.

Analysis led to the formula $C_6H_{11}NO$:—

			I.	II.	III.	IV.	V.
6C	72	63·71	63·64	62·78	63·62	63·93	„
11H	11	9·73	10·47	10·12	10·06	9·96	„
N	14	12·38					13·25
O	16	14·18					
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I. Extracted by ether from preparation by first method.

II. Leucimide prepared by hydrochloric acid as above related from leucine extracted from human liver, gave the following results :—0·2338 grm. burned with copper oxide yielded 0·5384 grm. CO₂ and 0·2132 H₂O, equal to 62·78 per cent. of C. and 10·12 per cent. of H.

III. The matter was thereupon recrystallised from alcohol, when it assumed larger forms, and was free from smell and colour; 0·16 grm. burned with oxide of copper and metallic copper in the fore part of the tube, gave 0·3734 grm. CO₂, equal to 0·1018 grm., or 63·62 per cent. C, and 0·1457 grm. H₂O, equal to 0·0161 grm., or 10·06 per cent. H.

IV. 0·318 grm. gave 0·7456 grm. CO₂, equal to 0·2033 or 63·93 per cent. of C, with 0·286 grm. H₂O equal to 0·0317 grm., or 9·96 per cent. H.

V. After the preparation used in analyses III. and IV. had been crystallised, the mother-liquor was again condensed, and a last preparation obtained, which was used for the nitrogen determination. Of this 0·2556 grm., when burned with soda-lime gave 0·2387 grm. Pt., equal to 0·0337 grm., or 13·25 per cent. nitrogen.

Theoretical Considerations.—Leucimide, like leucine and leucic acid, may be supposed to contain a radical leucyl, C₆H₁₀O, homologous with lactyl. Its formation from leucine may be represented by the equation—

The amylamine is due to the reaction—

While the leucine employed in the reaction should lose theoretically about 14 per cent. of weight by water, it actually loses

upwards of 20. About six per cent. are therefore lost in the collateral decompositions detailed above.

Leucinide was first observed by Bopp (Ann. Ch. Pharm. lxi, 28), afterwards by Hesse and Limpricht (*ibid*, cxvi, 201) and by Erlenmeyer and Schöffner (Verhandl. des Nat. Hist. med. Vereins zu Heidelberg, vol. 2, and Zeitschr. f. Chem. and Pharm. Erlangen, ii, 315), and by the latter three observers termed the nitrile of leucic acid. Kohler (Ann. Ch. Pharm., cxxxiv, 367) afterwards obtained it from synthetically prepared leucine, and the above observations are confirmatory of his results, and of the identity of synthetical leucine from valeraldehyde and hydrocyanic acid with that obtained from albuminous substances by sulphuric acid.

The circumstances under which leucinide is formed in the latter process are questionable. That it is not formed by the action of alcohol and lime upon the leucine mixture, as was probable from the manner in which it had been obtained by Bopp, but is a contemporary product of leucine and tyrosine, I have shown by extracting it repeatedly from the freshly-prepared leucine mixture. It may possibly be due to the prolonged influence of sulphuric acid upon already formed leucine in concentrated boiling mixtures.

One of the remarkable properties of leucinide is its indifference towards acids and alkalis. Although constructed upon the type ammonia, the properties therefrom derived are almost entirely lost. It resembles in this respect the secondary amides of the univalent acid radicals, such as acetyl. Diacetamide, $\left. \begin{array}{l} \text{C}_2\text{H}_3\text{O} \\ \text{C}_2\text{H}_3\text{O} \\ \text{H} \end{array} \right\} \text{N}$, imitates the construction of leucinide, by containing

two univalent acetyl groups in place of the two atoms of hydrogen which in leucinide are replaced by the bivalent leucyl, and, like leucinide, diacetamide has lost the power of combining with acids. Monacetamide, $\left. \begin{array}{l} \text{C}_2\text{H}_3\text{O} \\ \text{H}_2 \end{array} \right\} \text{N}$, on the other hand, retains the properties of a weak ammoniacal base.

When leucinide is extracted from the leucine mixture, it is frequently mixed with a considerable amount of a substance rich in sulphur. It is difficult to decide whether the sulphur of this substance is a residue of the original sulphur contained in the albuminous substance, a product of the influence of sul-

phuric acid and of the prolonged influence of alkali upon the mixture containing sulphates during evaporation, or of the sulphuretted hydrogen by which the excess of lead employed for purification has been removed from the mixture. I have isolated this sulphur compound on several occasions. It differs from leucimide by its greater solubility in ether, and by forming larger leaves of crystals of the aspect of mother-of-pearl. It yields a black precipitate on boiling with a solution of lead in caustic alkali, and in one analysis it contained 19.9 per cent. of sulphur; it decomposes during drying above 80° C., and when a concentrated solution in alcohol is heated beyond saturation, it forms brown drops of resin, a product of decomposition. This substance, which I have termed thiotherine, can therefore not be mistaken for leucimide, and can be separated from it by boiling with caustic potash, which leaves leucimide unchanged and crystalline.

By treatment of an alcoholic or ethereal solution of leucimide with sulphuretted hydrogen gas, this compound is not obtained. The great mass of leucimide crystallises unchanged, and free from sulphur; but a small quantity of a sulphur-compound is always formed, which has the odour of garlic, and adheres to the unchanged leucimide with great pertinacity. The compound is extremely volatile. It is possibly a sulphuretted aldehyde, and requires further examination. When to an alcoholic saturated solution of leucimide tincture of iodine is added, and the mixture boiled, slight discoloration ensues. Hydriodic acid is developed in small quantity. The mixture, after addition of excess of caustic potash and much water, deposits white crystals.