Mono-iodotyrosine

By C. R. HARINGTON AND ROSALIND V. PITT RIVERS, *National Institute for Medical Research, Hampstead, N.W. 3*

(Received 3 August 1944)

3:5-Di-iodotyrosine has been known for many years as a natural product, having been isolated from natural iodine-containing proteins such as gorgonin (Drechsel, 1895) and thyroglobulin (Harington & Randall, 1929); the process of isolation has usually involved alkaline hydrolysis so that the di-iodotyrosine obtained has been racemized, but the optically active compound has also been isolated by enzymic hydrolysis of thyroglobulin (Harington & Randall, 1931). The *dl*-compound has further been obtained by alkaline hydrolysis of artificially iodinated proteins (Oswald, 1911; Ludwig & von Mutzenbecher, 1939). Both the natural optically active di-iodotyrosine and the *dl*-amino-acid have been prepared synthetically by iodination of *l*- and *dl*-tyrosine respectively.

In contrast with this extensive information relating to di-iodotyrosine, references to mono-iodotyrosine have only recently appeared in the literature. In the course of their work on iodinated proteins Ludwig & von Mutzenbecher (1939) describe the isolation of mono-iodotyrosine from iodinated casein. The compound was obtained from the mother liquors of the fraction of the products of alkaline hydrolysis which contained di-iodotyrosine, after separation of the latter; its constitution was established by elementary analysis and by the facts that it gave 3:5-di-iodotyrosine on treatment with 1 mol. of iodine in alkaline solution and tyrosine on catalytic de-iodination.

More recently Herriott (1941-2) has given an account of the isolation of mono-iodotyrosine from partially iodinated crystalline pepsin. Herriott's description of the compound differs in several respects from that given by Ludwig & von Mutzenbecher. Thus his preparation separates from water in diamond-shaped crystals which are anhydrous, whilst that of the German authors is described as forming whetstone-shaped needles with $1\text{H}_2\text{O}$; in both cases the compound is stated to give the colour reaction with nitrous acid and ammonia which is characteristic of thyroxine and di-iodotyrosine, and Herriott states that the Millon reaction is also positive. Ludwig & von Mutzenbecher make no mention of optical activity; Herriott claims that his compound has $[\alpha]_D^{25} - 8.8^\circ$, an observation which is very surprising since the method of isolation from

iodinated pepsin involves prolonged hydrolysis with concentrated barium hydroxide; such treatment should lead to complete racemization of any tyrosine derivative. In any case, since the preparation of Ludwig & von Mutzenbecher was isolated from a similar hydrolysate the difference between it and that of Herriott cannot reasonably be ascribed to difference in stereochemical configuration.

During the working up of the mother liquors from an experiment on the oxidation of di-iodo-*l*-tyrosine (shortly to be described in another paper), in which not very highly purified di-iodotyrosine was employed as starting material, we ourselves encountered a crystalline amino-acid which analyzed for mono-iodotyrosine but did not agree entirely in its properties with either of the compounds described by Herriott and by Ludwig & von Mutzenbecher. Since we were anxious to establish the identity of our product without doubt we decided to attempt the synthesis of mono-iodo-*l*- and *dl*-tyrosine by unequivocal methods. This we have accomplished by conversion of *l*- and *dl*-tyrosine respectively into the corresponding 3-amino derivatives (Waser & Lewandowski, 1921), diazotization of the latter and decomposition of the diazonium salts with iodide.

3-Iodo-*l*-tyrosine obtained in this manner crystallizes in blunt needles or lozenge-shaped crystals which are anhydrous; in *N*-hydrochloric acid it has $[\alpha]_D^{20} - 4.4^\circ$; in both these respects it is identical with the compound isolated by us as a by-product from impure di-iodo-*l*-tyrosine and is different from the preparations both of Herriott and of Ludwig & von Mutzenbecher.

Synthetic 3-iodo-*dl*-tyrosine on the other hand forms triangular or diamond-shaped plates of which the corners are frequently rounded so that they appear with the outline of a whetstone; the crystals contain $1\text{H}_2\text{O}$ which cannot be removed without decomposition and loss of iodine. The compound thus corresponds closely with that described by Ludwig & von Mutzenbecher.

We therefore conclude that the compound isolated by the latter authors was unquestionably 3-iodo-*dl*-tyrosine; on the other hand we have found nothing resembling the substance described by Herriott and we are unable to offer any explanation of his results.

EXPERIMENTAL

A. 3-Iodo-*l*-tyrosine

3-Amino-*l*-tyrosine (Waser & Lewandowski, 1921) (6 g.) was dissolved in cold H_2SO_4 (45 ml. of 1:5); the solution was diluted with water (60 ml.), cooled to -2° and diazotized by addition of a solution of $\text{Ba}(\text{NO}_3)_2$ (3.51 g.) in water (60 ml.), the temperature being maintained between 0° and -2° . The solution was then poured into boiling concentrated aqueous KI (25 g. : 5 equiv.) containing copper bronze in suspension; the addition was made at such a rate that ebullition was not interrupted, instantaneous decomposition of the diazonium salt being essential for good results.

The mixture was filtered hot and the filtrate evaporated under diminished pressure; on taking up the residue in hot water and cooling, a first crop of 3.4 g. was obtained; a second crop, contaminated with salt, was isolated by concentration of the mother liquor. Recrystallization of the united crops from water (80 ml.; charcoal) gave 4.4 g. (47% of theory) of pure material.

The pure 3-iodo-*l*-tyrosine dissolved in about 15 parts of boiling water from which it crystallized in blunt needles or lozenge-shaped crystals. It had m.p. $204-206^\circ$ (decomp.). (Found: C, 35.6; H, 3.8; N, 4.8; I, 41.1%. $\text{C}_9\text{H}_9\text{O}_3\text{NI}$ requires C, 36.1; H, 3.7; N, 4.6; I, 41.4%.) In N-HCl the amino-acid had $[\alpha]_D^{20} - 4.4^\circ$ ($c=5$).

In order to ensure that no racemization had occurred during the synthesis, part of the 3-iodotyrosine was converted into tyrosine by shaking in alkaline solution in an atmosphere of hydrogen with palladized strontium carbonate as catalyst; 1 g. of 3-iodo-*l*-tyrosine ($[\alpha]_D^{20} - 4.4^\circ$) gave 0.56 g. (95% of theory) of tyrosine having $[\alpha]_D^{20} - 12.4^\circ$ ($c=5$ in N-HCl).

B. 3-Iodo-*dl*-tyrosine

3-Nitro-*dl*-tyrosine. *dl*-Tyrosine (8 g.) was suspended in water (30 ml.) and treated with HNO_3 (21.4 ml. of sp. gr. 1.42), the temperature being kept below 25° . The nitrate of the product crystallized rather slowly; after being kept for some time at 0° it was collected and suspended in a small amount of water; ammonia (sp. gr. 0.880) was added until the suspension was neutral and the whole was brought to the boil, more ammonia being added as necessary to maintain neutrality to Congo Red. After cooling, the amino-acid was collected, washed and dried. Yield, 7.25 g. (72.5% of theory).

For analysis it was recrystallized by boiling the solution in 0.1 N-HCl with charcoal and neutralizing the hot filtrate with ammonia; it separated in yellow prismatic needles, m.p. $214-215^\circ$ (decomp.). (Found: N (Dumas), 12.1%; $\text{C}_9\text{H}_{10}\text{O}_3\text{N}_2$ requires N, 12.4%.)

3-Amino-*dl*-tyrosine. This was prepared from 3-nitro-*dl*-tyrosine precisely as described by Waser & Lewandowski (1921) for the *l*-isomeride; 7.25 g. of the nitro compound gave 3.02 g. (48% of theory) of 3-amino-*dl*-tyrosine, crystallizing from water in colourless stumpy prisms having m.p. 288° (decomp.). (Found: N, 14.5%; $\text{C}_9\text{H}_{11}\text{O}_3\text{N}_2$ requires N, 14.3%.)

3-Iodo-*dl*-tyrosine. The diazotization and decomposition of the diazonium salt were carried out as described above for the *l*-isomeride; the yield of re-crystallized 3-iodo-*dl*-tyrosine was 44% of theory. The amino-acid crystallized from water in triangular or diamond-shaped plates, often with rounded corners, containing $1\text{H}_2\text{O}$ and having m.p. $200-201^\circ$ (decomp.). The solubility was similar to that of the *l*-isomeride. (Found: C, 33.7; H, 3.9; N, 4.4; I, 38.8%. $\text{C}_9\text{H}_{10}\text{O}_3\text{NI} \cdot \text{H}_2\text{O}$ requires C, 33.2; H, 3.7; N, 4.3; I, 39.1%.)

Colour reactions of mono-iodotyrosine

In contrast with di-iodotyrosine, mono-iodotyrosine gives the Millon reaction with intensity. On the other hand, we are not able to agree with Ludwig & von Mutzenbecher or with Herriott that it gives the colour reaction with nitrous acid and ammonia which is characteristic of thyroxine and which is also given, though with less intensity, by di-iodotyrosine. If 3-iodotyrosine is boiled with aqueous-ethanolic HCl and a large excess of nitrite, an orange-red colour is indeed obtained on addition of excess of ammonia; under the conditions commonly employed for the test, however, no trace of red colour is to be observed.

SUMMARY

1. 3-Iodo-*l*-tyrosine and 3-iodo-*dl*-tyrosine have been synthesized, and their properties have been compared with those of reputed preparations of mono-iodotyrosine, accounts of which have recently appeared.

2. It is concluded that the compound obtained by Ludwig & von Mutzenbecher (1939) from iodinated casein was in fact 3-iodo-*dl*-tyrosine; that isolated by Herriott (1941-2) from iodinated pepsin does not correspond with either of the synthetic compounds and its nature remains in doubt.

REFERENCES

- Drechsel, E. (1895). *Z. Biol.* **33**, 85.
 Harington, C. R. & Randall, S. S. (1929). *Biochem. J.* **23**, 373.
 ——— (1931). *Biochem. J.* **25**, 1032.
 Herriott, R. (1941-2). *J. gen. Physiol.* **25**, 185.

- Ludwig, W. & von Mutzenbecher, P. (1939). *Hoppe-Seyl Z.* **258**, 195.
 Oswald, A. (1911). *Hoppe-Seyl Z.* **70**, 310; **71**, 200; **74**, 290.
 Waser, E. & Lewandowski, M. (1921). *Helv. chim. Acta*, **4**, 657.