L.—*The Constitution of Allantoin and Allied Substances.*

By HENRY DRYSDALE DAKIN.

THE customary formula for allantoin (IV), based largely on Grimaux's synthesis from glyoxylic acid and carbamide, contains an asymmetric carbon atom, and hence allantoin would be expected to be capable of existence in optically active forms. Mendel and the author (*J. Biol. Chem.*, 1910, **7**, 153) have shown that naturally occurring allantoin, isolated from urine with a minimum of chemical manipulation, is optically inactive. Furthermore, attempts to resolve allantoin into an active component by bacterial action gave negative results.

The natural occurrence of optically inactive substances containing asymmetric carbon atoms is rare, so that it was of interest to endeavour to determine the cause of the inactivity of allantoin. Now allantoin, according to Grimaux's formula, is represented as a substituted hydantoin, and the author (*Amer. Chem. J.*, 1910, **44**, 48) has shown that optically active hydantoins of the most varied kind, containing a hydrogen atom linked to carbon (I), undergo racemisation with extraordinary ease, at room temperature, especially but not exclusively in the presence of alkali. The cause of the racemisation was shown, apparently conclusively, to be due to tautomeric change of the keto-enol type, the enol form (II) being produced in low concentration with loss of asymmetry:

The validity of this assumption was emphasised by the fact that active methylethylhydantoin (III), a compound containing no labile hydrogen atom attached to carbon, and hence incapable of showing keto-enol tautomerism, did not undergo racemisation at all under conditions which would have racemised other hydantoins completely.

It appeared reasonable to ascribe the optical inactivity of the hydantoin derivative, allantoin, to the same cause as that which conditions the racemisation of other hydantoin compounds. The author therefore suggested that allantoin might exhibit tautomeric change of the keto-enol type, the formation of the enol form (V) being followed by loss of asymmetry:

Recently Titherley (T., 1913, **103**, 1336) has taken exception to this view, and suggested an alternative explanation, which will be referred to later. Titherley writes: "The tautomerism postulated by Mendel and Dakin is improbable, since it requires for its manifestation the influence of negative groups such as are absent in the allantoin molecule. Such keto-enolic tautomerism should, in fact, appear with consequent rapid racemisation in α -amino-acids, and similarly constituted compounds where it is not apparent, if Mendel and Dakin's theory were to be accepted." The author believes that Titherley's criticism cannot be sustained, for in the first place "the influence of negative groups such as are absent in the allantoin molecule" is certainly not necessary for the exhibition of keto-enol tautomerism, neither is the rapid racemisation of α -amino-acids a necessary corollary of our hypothesis, but rather the reverse.

Certain facts will illustrate the above points. The following optically active amino-acids, among others, have been converted successively into the corresponding carbamido-acids and hydantoins: alanine, aspartic acid, glutamic acid, leucine, α -aminophenyl-acetic acid (Dakin and Dudley, *J. Biol. Chem.*, 1914, **18**, 48), phenylalanine (Dakin and Dudley, *ibid.*, 1913, **17**, 29), and tyrosine:

The parent amino-acids, as is well known, are relatively resistant to racemisation, although prolonged boiling with alkali does effect a slow but perceptible racemisation as with most other com-

pounds*; on treating the active amino-acid with potassium cyanate, the active carbamido-acid is readily obtained without racemisation, and the optical stability of the product is about as great as that of the parent amino-acid. As soon as the active carbamido-acid is converted into the cyclic hydantoin by warming with dilute mineral acids, the optical stability of the product is at once impaired. In the first place, it appears that the actual conversion into hydantoin by acids is usually accompanied by some racemisation, so that the optical rotations for various active hydantoins described by the author can only be regarded as minimum values. In the case of the conversion of 1- α -carbamidophenylacetic acid ($[\alpha]_D -133.5^\circ$) into phenylhydantoin by boiling with 5 per cent. hydrochloric acid, the product was completely racemised, and the same tendency, but to a less degree, has been observed in several other cases. The activity of the hydantoins was in every case abolished by short treatment with dilute alkali, and the change proceeds rapidly to completion at room temperature. The hydantoins must be regarded as optically unstable compounds whenever their structure permits of keto-enol tautomerism.

The author considers that the assumption that allantoin would exhibit the behaviour common to other hydantoins appears well based, and that at present there is no evidence antagonistic to the idea that these compounds undergo keto-enol tautomeric change.

Titherley suggests that the inactivity of allantoin may be due to tautomerism of a different kind, and assumes the formation of a symmetrical bicyclic structure, the formula for which had been previously considered and rejected on chemical grounds by Biltz (*Ber.*, 1910, **43**, 1999)[†]:

The occurrence of tautomerism of this character is somewhat

* It appears not improbable that keto-enol tautomerism may be responsible for this change, especially as Dudley and the author have shown that minute amounts of alkylglyoxalbismnitrophenylhydrazones result from the digestion of α -amino- and α -hydroxy-acids with *p*-nitrophenylhydrazine. From a quantitative point of view, the reaction takes place only to a very slight extent (compare footnote, *T.*, 1914, 105, 2453).

[†] The only direct evidence in favour of this symmetrical formula is the fact that allantoin may be reduced to the bicyclic glycouril, as shown by Rheineck and confirmed by Biltz. This reaction can be equally well accounted for by the conventional formula for allantoin, and, moreover, the significance of the reaction is lessened by the fact that Biltz has shown that 3-methylallantoin, which is undoubtedly structurally similar to allantoin, on similar reduction yields carbamide and probably methylallantoin. It has not been possible to reconvert glycouril into allantoin, so that on the whole there seems no good reason at present for reconsidering the bicyclic formula for allantoin.

analogous to the tautomerism observed by Titherley in the case of the *O*-acyl and *N*-acyl derivatives of salicylamide, but so far as the author is aware has not been observed in any compounds even remotely related to allantoin.

Since tautomerism of the type suggested by Titherley, as well as that put forward by Mendel and the author, may occur in the case of allantoin, it appeared desirable if possible to submit the question to the test of a crucial experiment. With this end in view, the author has made a large number of attempts to resolve 3-methylallantoin (β -methylallantoin) and homoallantoin (pyruvil) into active components, but all have been unsuccessful.

EXPERIMENTAL.

3-Methylallantoin was prepared according to Fischer and Ach's direction (*Ber.*, 1899, **32**, 2746) by the oxidation of 7-methyluric acid with lead peroxide. The latter substance was obtained by the reduction with tin and hydrochloric acid of 7-hydroxy-methyluric acid prepared by the action of formaldehyde on sodium urate, as described by Grohmann (*Annalen*, 1911, **382**, 66). The yields throughout were satisfactory.

Homoallantoin was readily prepared according to Simon's method (*Compt. rend.*, 1901, **133**, 587) by heating homoallantoic acid, prepared by the action at room temperature of pyruvic acid on carbamide, with a small amount of water at 100°.*

Three types of experiments were made with each compound in the attempt to effect their resolution into active components.

(a) The substances were administered to rabbits, and the unchanged allantoin was isolated from the urine. Portions of a gram, each dissolved in warm water, were given by stomach tube twice a day. The unchanged allantoin was separated from the urine by direct crystallisation after successive treatment with lead acetate and hydrogen sulphide. The yield of substance recovered in each case amounted to about a quarter of that given, and was optically inactive. The substances were not toxic.

(b) Ehrlich's method (*Biochem. Zeitsch.*, 1914, **63**, 379) for the

* It is worthy of note that a solution of mercuric acetate with an excess of sodium acetate, such as Wiechowski uses for the quantitative precipitation of allantoin, produces no precipitate with solution of 3-methylallantoin. This fact is probably connected with Biltz's observation that the silver salt of allantoin on methylation yields 3-methylallantoin. It appears probable, therefore, that the mercury complex in Wiechowski's allantoin compound is attached to the nitrogen atom in position 3.

Homoallantoin, as expected, is precipitated by Wiechowski's reagent much in the same way as is allantoin, since the nitrogen in position 3 of homoallantoin is unsubstituted.

resolution of amino-acids by the use of actively fermenting yeast was employed without success. Ten grams of homoallantoin or 3-methylallantoin and 400 grams of sucrose were fermented at room temperature for four days with 200 grams of freshly-washed and pressed yeast. The yeast was removed by filtering through a Berkefeld filter and the sugar-free filtrate concentrated by distillation under diminished pressure, when 5—7 grams of each substance were recovered. The products were entirely inactive.

(c) Attempts to resolve the substances by the action of bacteria and moulds were made by growing the organisms in a suitable inorganic salt solution with 0.5 per cent. of the allantoin derivatives, both with and without the addition of 1 per cent. of sucrose. An actively growing organism isolated from urine undergoing ammoniacal fermentation was chosen for some experiments, since it was thought that a urea-decomposing organism might be specially adapted for the decomposition of the ureides under examination. Other experiments were made with mixed organisms obtained from faeces, since allantoin has been shown by Givens (*J. Biol. Chem.*, 1914, **18**, 417) to be readily decomposed by intestinal organisms. In addition, experiments were made with various types of moulds. The organisms grew well, but the recovered allantoin was inactive.

Attempts to prepare optically active homoallantoin from active methylhydantoin were unsuccessful. The action of bromine and carbamide on methylhydantoin under similar conditions to those described by Siemonsen (*Annalen*, 1904, **333**, 101) for the synthesis of allantoin gave negative results. Attempts to reduce Franchimont and Klobbie's nitromethylhydantoin (*Rec. trav. chim.*, 1888, **7**, 13) to aminohydantoin and then to convert the latter into hydantoin by means of potassium cyanate were equally fruitless.

In the course of these experiments some new data were obtained relating to the α -carbamidopropionic acids and methylhydantoins. The active substances have not been previously prepared.

l- α -Carbamidopropionic Acid, $\text{CH}_3\cdot\text{CH}(\text{NH}\cdot\text{CO}\cdot\text{NH}_2)\cdot\text{CO}_2\text{H}$.—Optically pure *d*-alanine (5 grams) and potassium cyanate (10 grams) were heated on the water-bath in an open dish with 20 c.c. of water for one hour. The concentrated solution was made just acid to Congo-red with hydrochloric acid. The carbamido-acid crystallised out in good yield, and after recrystallisation from water the melting point was constant. The pure substance is readily soluble in hot water, but requires about seventy parts of cold water for solution. It crystallises in clear, hexagonal prisms, sintering at 195° and melting at 198 — 200° . (Found, $\text{N}=21.0$. $\text{C}_4\text{H}_8\text{O}_3\text{N}_2$ requires $\text{N}=21.2$ per cent.)

The rotation was observed in aqueous solution :

$$c = 1.281; l = 2.2 \text{ dm.}; \alpha = 0.27^\circ; [\alpha]_D^{20} = -9.6^\circ.$$

Another preparation under similar conditions gave $[\alpha]_D = -9.4^\circ$.

l-Methylhydantoin, $\text{CH}_3 \cdot \text{CH} \begin{smallmatrix} \text{NH} \cdot \text{CO} \\ \text{CO} \cdot \text{NH} \end{smallmatrix} - \text{On}$ boiling *l*- α -carbamidopropionic acid (3 grams) with 50 c.c. of water and 9 c.c. of concentrated hydrochloric acid for two hours, the hydantoin is readily obtained. It is very readily soluble in water, and is left as a crystalline meal on evaporation of the acid. After recrystallisation from a little water, the substance melts at $175\text{--}177^\circ$ without decomposition.

Found: $\text{N} = 24.7$.

$\text{C}_4\text{H}_6\text{O}_2\text{N}_2$ requires $\text{N} = 24.6$ per cent.

The substance is strongly levorotatory in aqueous solution. The actual rotation calculated was $[\alpha]_D^{20} = -50.6^\circ$ for a 2.836 per cent. solution in water. This can only be regarded as a minimum value, since racemisation is apt to accompany the formation of the hydantoin. The active methylhydantoin is rapidly racemised by digestion with dilute alkali at room temperature.

Inactive α -Carbamidopropionic Acid.—This substance was prepared as described for the active compound. It has a great tendency to form supersaturated solutions, so that recrystallisation is sometimes difficult. After three crystallisations from water the substance sinters slightly at 182° , and melts sharply at 185° . (Found, $\text{N} = 21.2$. Calc., $\text{N} = 21.2$ per cent.) Apart from the melting point, which has been given as 155° , the properties of the substance agree with those described by Urech (*Annalen*, 1873, **165**, 99) and by Heintz (*Annalen*, 1873, **169**, 120). The cause of the discrepancy in the melting-point observations is obscure, but it is possible that the preparations of Urech and Heintz had undergone partial conversion into methylhydantoin.

Inactive α -Methylhydantoin.—The substance was prepared in the same way as the active compound. It may be extracted by ether from its aqueous solution in a few hours in a continuous extractor. It crystallises in clear, hard prisms, melting sharply at 150° without decomposition. The melting point of the substance is stated to be 125° and 140° by Urech and by Heintz respectively. The other properties observed agree with the earlier descriptions. On boiling with dilute barium hydroxide and then removing the barium quantitatively with sulphuric acid, the hydantoin was re-converted into α -carbamidopropionic acid, which crystallised out on concentrating the solution, and melted at 185° .

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