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THE STEREOCHEMICAL FORMULAS OF THE HYDROXYPROLINE AND ALLOHYDROXYPROLINE ENANTIOMORPHS AND SOME RELATED SUBSTANCES

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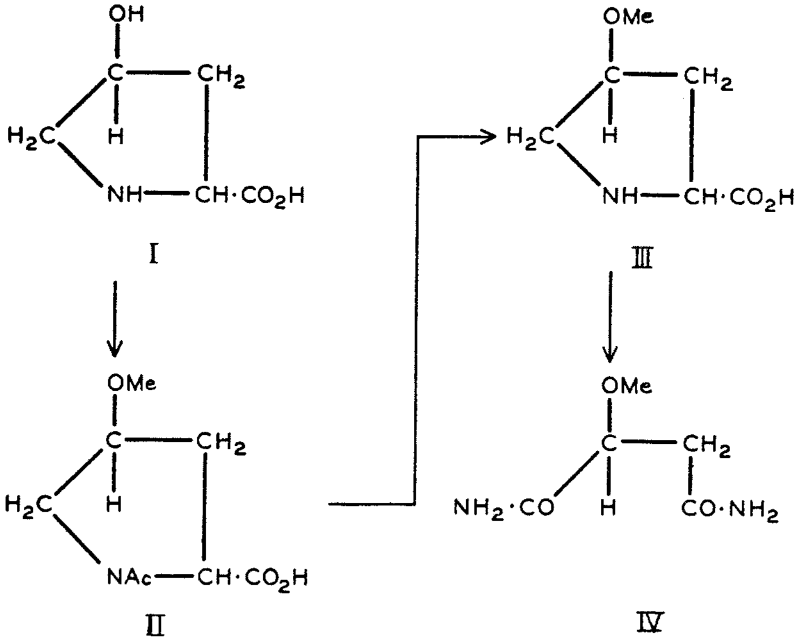
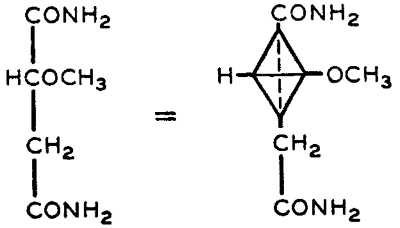
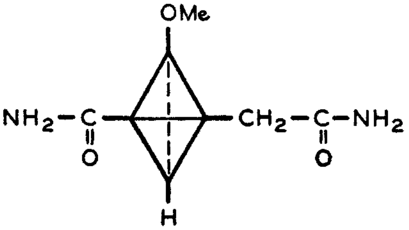
In 1945 one of us reported (1) experimental data of a conclusive nature which disclose the configuration of the asymmetric γ-carbon atom of the naturally occurring α-amino acid hydroxyproline and its enantiomorph, and supplementary data which allow extension of these determinations to the enantiomorphs of allohydroxyproline. Starting with natural hydroxyproline ($[\alpha]_D$ $-76.3°$), its O-methyl ether was prepared and from this substance there was obtained in a series of degradation reactions the crystalline dextrorotatory diamide of methoxysuccinic acid, the configuration of which had been previously established as



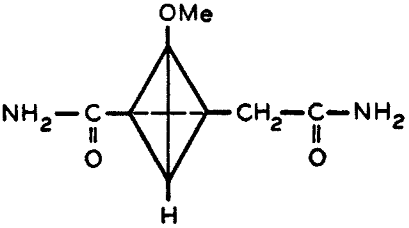


in the configurational system that was originally instituted by Emil Fischer. There was then deduced from these experimental data a perspective partial stereo-formula of natural hydroxyproline through the sequence of formulas shown here as I–IV.

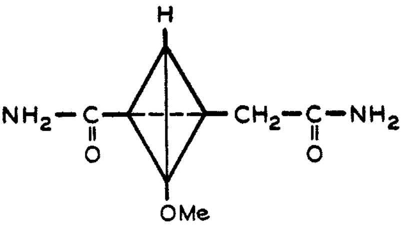
Subsequent to the publication of formula I it has been recognized by each of us independently that this formula does not represent correctly the configuration of the asymmetric γ-carbon atom. If one constructs the customary mechanical model of I by the use of a tetrahedron for its asymmetric γ-carbon atom it becomes apparent that IV must be interpreted as



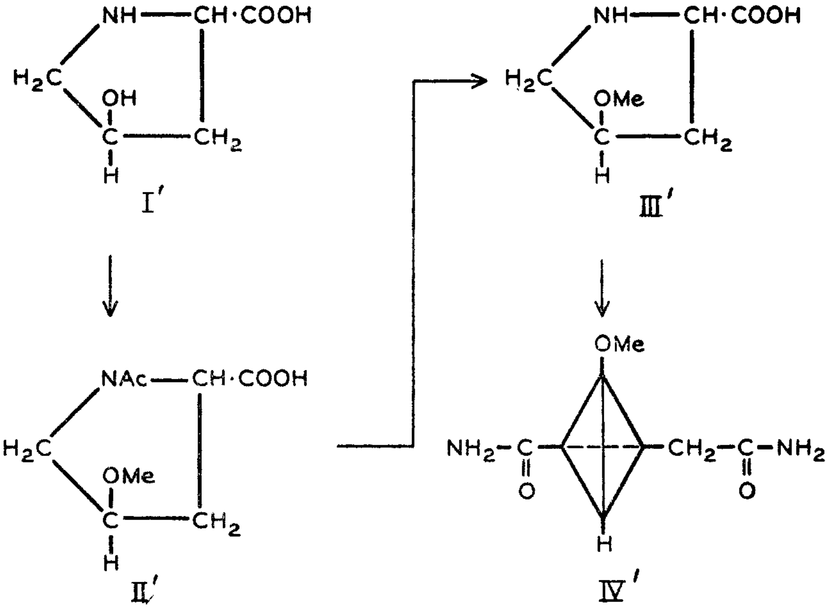
which is not the stereo-formula for the dextrorotatory diamide of methoxysuccinic acid in the Fischer system; the correct stereo-formula for this dextrorotatory diamide is



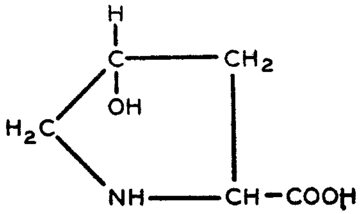
and formula IV really represents the levorotatory diamide, the formula of which by the Fischer conventions is



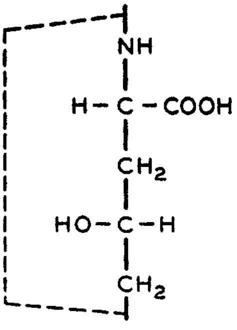
If one wishes to express the sequence I–IV in the universally accepted conventional system of Fischer, the development is correctly shown by the formulas I′–IV′. It will be seen that formulas I and I′ are enantiomorphous as respects the


I′ II′ III′ IV′

asymmetric γ-carbon atom, a relationship that may be visualized more readily if one turns the mechanical model of I′ so that its perspective picture is I″; in I″ the ring lies in the plane of the paper, the OH group lies below this plane and the


I″

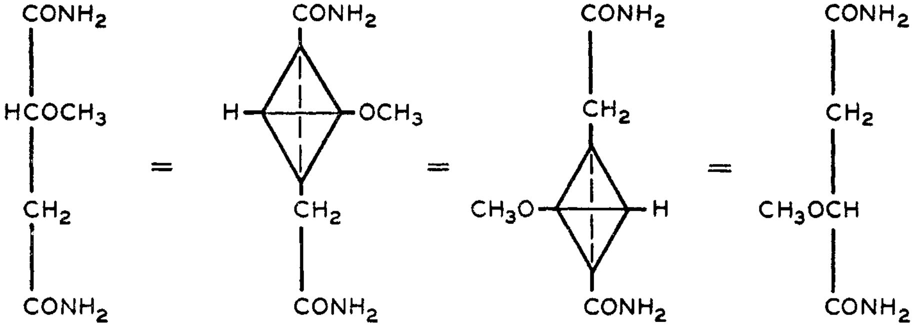
H attachment to the γ-carbon atom lies above this plane. In a review article (2) of recent date the new formula V for natural hydroxyproline was deduced and it was stated that "this formula in which the plane of the ring is perpendicular


V

to the plane of the paper shows very clearly the *trans* position of the two substituents [OH and COOH] in hydroxyproline." This formula V conforms with the formula I′ as respects the asymmetric γ-carbon atom provided that the reader observes the usual convention that the dotted line lies below the plane of the paper.

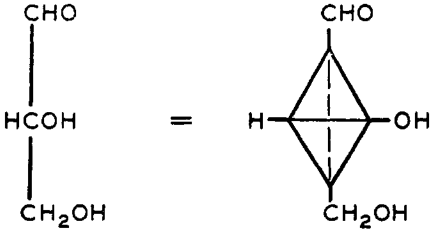
We believe it advisable for the sake of full clarity that the development of the stereo-formula for natural hydroxyproline ($[\alpha]_D$ −76.3°) should be presented anew, with particular attention at all steps to the observance of the two conventions that were established by Emil Fischer for writing the stereo-formulas of organic chemistry in a plane.[1]

The stereo-formula of the dextrorotatory diamide of methoxysuccinic acid is VI, which may also be written as VII, VIII or IX.



VI VII VIII IX

These equivalent formulas are all of the Fischer system and they preserve his two conventions. The first convention specifies the mechanical model that is represented by such a formula as VI; a straight line joining the connecting apices of the four carbon atoms (tetrahedra) lies in the plane of the paper and the H and OCH_3 attachments to the asymmetric carbon atom lie above the plane of the paper. Formula VII is a representation of the model in such a way that this first convention is indicated graphically; the dotted (invisible) back edge of the tetrahedron lies in the straight line of the four carbon atoms in the plane of the paper and the visible edge connecting H and OCH_3 lies above the plane of the paper. In present day practice Fischer's second convention selects as reference substance the



X XI

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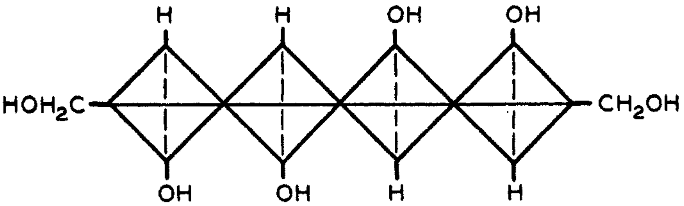
[1] These conventions are the subject of a review article by one of us (H) in *Advances in Carbohydrate Chem.*, **3,** 1 (1948).

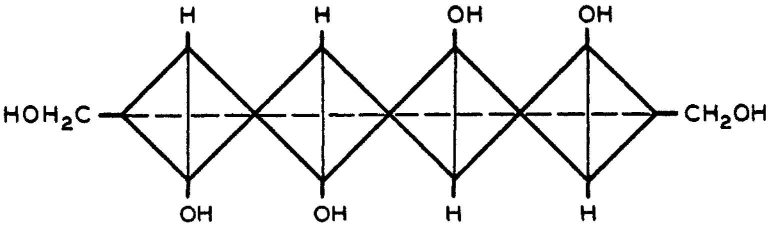
dextrorotatory enantiomorph of glyceraldehyde, arbitrarily assigns to it the configuration X and designates it as $\mathrm{D_g}$-glyceraldehyde.

The subscript g in the prefix $\mathrm{D_g}$ indicates that the D is being used in the sense of carbohydrate nomenclature. Later, the prefixes $\mathrm{D_s}$ and $\mathrm{L_s}$ will appear in designation of the enantiomorphs of some α-amino acids. The subscript g stands for glyceraldehyde as the refererence substance of the carbohydrate nomenclature and s is the analogous abbreviation for serine, the reference substance of the α-amino acid system of nomenclature. The conclusive correlation of the enantiomorphs of these two reference substances through precise chemical transformations depends upon the following observations: (a) carbon atom 2 in chitosamine was correlated in configuration with one of the enantiomorphs of glyceraldehyde by Haworth, Lake, and Peat (3), who proved that chitosamine is 2-desoxy-2-amino-D-glucose; (b) carbon atom 2 of chitosamine was correlated in configuration with one of the enantiomorphs of alanine by Wolfrom, Lemieux, and Olin (4); the enantiomorphs of alanine and serine were correlated by Fischer and Raske (5). The need for some method of distinguishing between the D and L nomenclatures of carbohydrates and α-amino acids in cases where confusion can arise, and the use of g and s subscripts to meet this need, were proposed by one of us (H) in January 1947; see a report by H. B. Vickery, *J. Biol. Chem.*, **169**, 242 (1947) and *Chem. Eng. News*, **25**, 1365 (1947). The D and L symbols are often the same in the carbohydrate and α-amino acid nomenclatures but this is not always the case; thus $\mathrm{D_g}$-glucosaminic acid is a $\mathrm{D_s}$-α-amino acid but $\mathrm{D_g}$-mannosaminic acid is an $\mathrm{L_s}$-α-amino acid, and natural threonine is an $\mathrm{L_s}$-α-amino acid although it is configurationally related closely to $\mathrm{D_g}$-threonic acid.

The earliest use that is known to us of graphic formulas of the type of VII and VIII to represent the two Fischer conventions is the formula of $\mathrm{D_g}$-mannitol that was published by L. Maquenne in his textbook "Les Sucres et Leurs Principaux Dérivés" (Carré and Naud, Paris, 1900). Maquenne's formula (p. 13) is



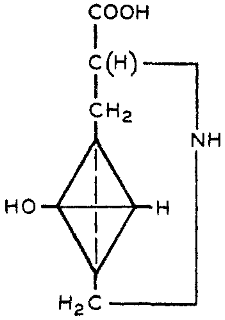
but it is incorrectly drawn as it does not follow Fischer's first convention and the mechanical model which it represents is really that of $\mathrm{L_g}$-mannitol. The correct formula for $\mathrm{D_g}$-mannitol is



which preserves Fischer's first and second conventions.

The configuration VIII for the dextrorotatory diamide of methoxysuccinic acid leads to the partial stereo-formula XII for natural hydroxyproline ($[\alpha]_D$ −76.3°);

in this formula the arrangements for the asymmetric γ-carbon atom are specified through Fischer's two conventions but the arrangements for the asymmetric α-carbon atom are left unspecified. To change formula XII to a perspective formula XIV of the type that was introduced by Drew and Haworth (6) for cyclic carbohydrate structures, one observes with a mechanical model that the tetrahedron representing the α-carbon atom must be turned 120° in order that the ring may be represented as in a plane perpendicular to the plane of the paper and lying below the paper; this is represented in XIII. Formula XIV is then readily visualized as a perspective view of XIII. Referring back to formulas I and I′ one recognizes that XIV is in agreement with I′ but not with I.



XII

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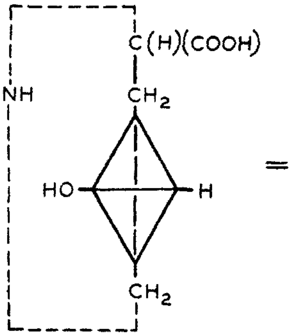

XIII

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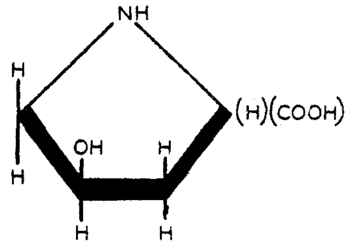
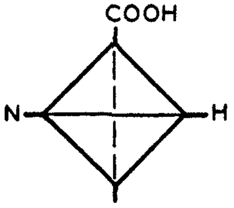


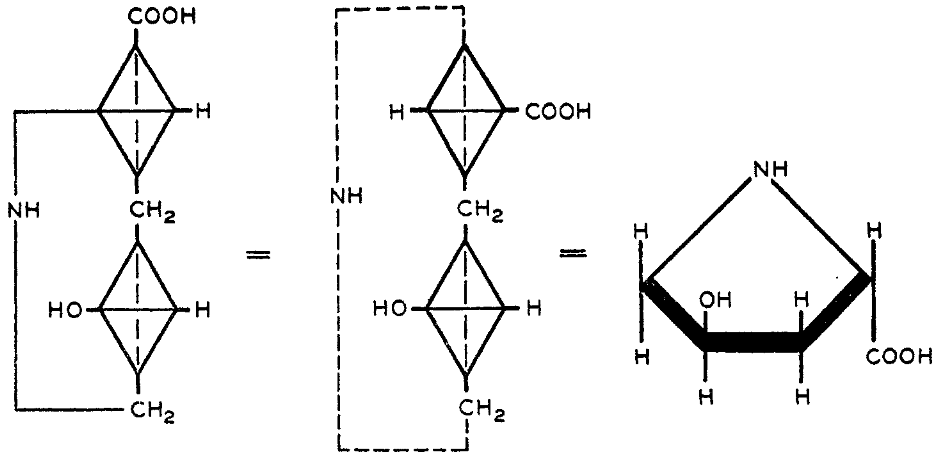
XIV

Formulas I′ and XIV leave unspecified the stereo arrangement for the α-carbon atom. The article (1) by one of us (N) summarizes the strong evidence from several earlier sources that in natural hydroxyproline ($[\alpha]_D$ −76.3°) this atom has the L$_s$ configuration XV, namely that of L$_s$-serine. When this arrangement is supplied to I′ and XII, the full configurational formula of natural L$_s$-hydroxyproline becomes XVI in the Fischer system, and the equivalent in the Haworth perspective representation becomes XVIII, through XVII. Formulas V and XVII are in agreement.



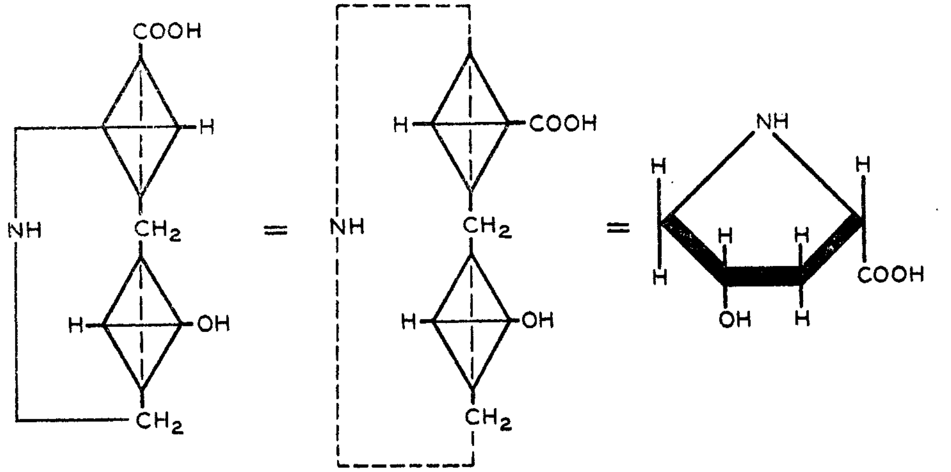
XV

In the research (1) by one of us (N) L$_s$-hydroxyproline (XVIII) was converted to one of the enantiomorphs of its diastereomer by a Walden inversion of the arrangement of the γ-carbon atom; this enantiomorph is accordingly named L$_s$-



XVI XVII XVIII

allohydroxyproline ($[\alpha]_D$ −58.1°), and its stereo-formula is obviously XIX and the equivalent XXI, through XX.



XIX XX XXI

The stereo-formulas that have now been established for the enantiomorphs of hydroxyproline and allohydroxyproline through the experimental data (1) of one of us (N) regarding the γ-carbon atom and the earlier evidence concerning the α-carbon atom are summarized in Table I.

The perspective formulas of the table can be extended to several other substances, including some alkaloids.

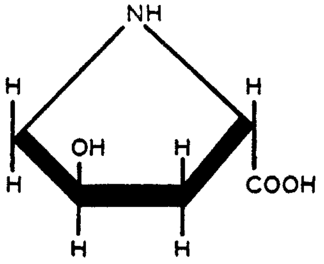
L_s-Hydroxyproline was converted by Kaneko (9) to a γ-chloroproline which could be reduced to natural (levorotatory) proline, which accordingly can be assigned the formula XXVI. The formula of its enantiomorph becomes XXVII.

The earlier evidence that natural proline and natural hydroxyproline are sterically related to L_s-serine involved some uncertainty because of the fact that

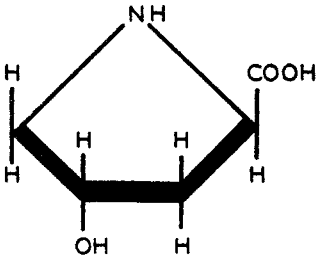
TABLE I

The Enantiomorphs of Hydroxyproline and Allohydroxyproline

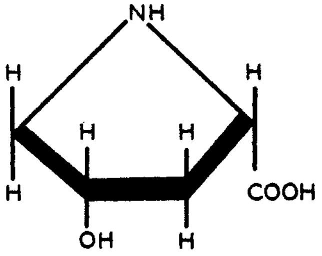
$\mathrm{L_s}$-Hydroxyproline, $[\alpha]_D$ −76.3° (XXII) (Occurs in proteins)



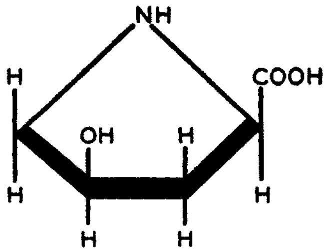
$\mathrm{D_s}$-Hydroxyproline, $[\alpha]_D$ +75.2° (XXIII) (Natural occurrence unknown)

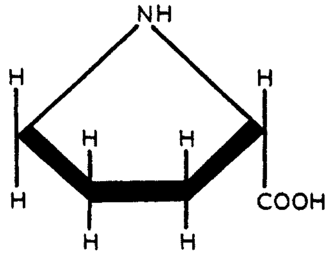


$\mathrm{L_s}$-Allohydroxyproline (7), $[\alpha]_D$ −58.1° (XXIV) [Occurs in phalloidine, a toxic peptide from *Amanita phalloides* (8)]

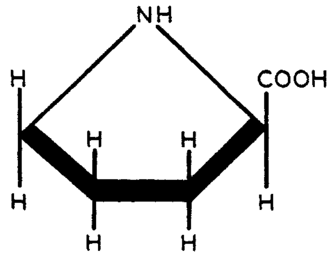


$\mathrm{D_s}$-Allohydroxyproline (7), $[\alpha]_D$ +58.6° (XXV) (Natural occurrence unknown)





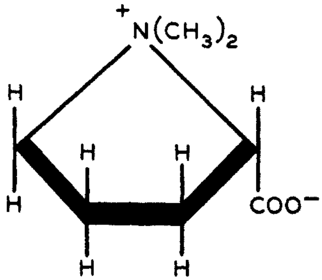
XXVI
$\mathrm{L_s}$-Proline
(Levorotatory, occurs in proteins)



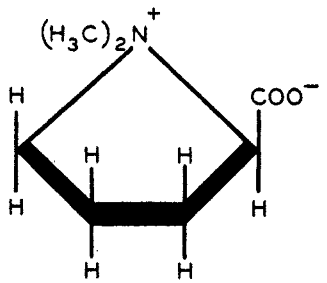
XXVII
$\mathrm{D_s}$-Proline
(Dextrorotatory, natural occurrence unknown)

the nitrogen atom of proline is a member of the pyrrolidine ring. The recent experimental work of Karrer and Portmann (10b) in chemically correlating natural proline with natural glutamic acid, which possesses no ring and is believed on very strong evidence to be sterically related to $\mathrm{L_s}$-serine, gives a more secure foundation to the older inference that natural proline is $\mathrm{L_s}$-proline.

The levorotatory enantiomorph of stachydrine has been correlated sterically with $\mathrm{L_s}$-proline (10); the perspective formula for this enantiomorph thus becomes XXVIII and that of its mirror image becomes XXIX.



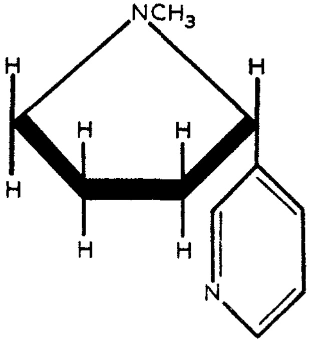
XXVIII
$\mathrm{L_s}$-Stachydrine
(Levorotatory hydrochloride)



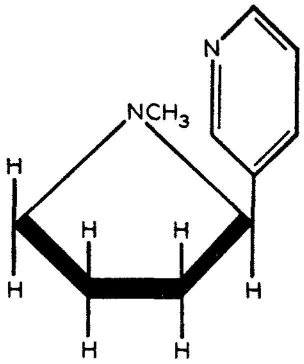
XXIX
$\mathrm{D_s}$-Stachydrine

Since the naturally occurring levorotatory enantiomorph of nicotine has been shown by Karrer and Widmer (10a) to be sterically related to $\mathrm{L_s}$-proline, natural nicotine is to be given the perspective formula XXX and its enantiomorph the formula XXXI.

The mechanical model that is represented by XXX is that of natural nicotine through Fischer's second convention (*i.e.*, that XI is the model of $\mathrm{D_g}$-glyceraldehyde and in consequence XV the analog for $\mathrm{L_s}$-serine). While there is thus no ambiguity with respect to the correlation of actual models, the use of an L or D symbol for natural nicotine becomes a matter of convention in nomenclature. Nicotine possesses no carboxyl or carbonyl group and thus one must select some other similarity between the formulas for nicotine and serine (or glyceraldehyde) as an arbitrary basis for L and D nomenclature. Fortunately, the selection in this



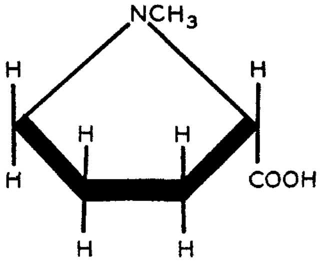
XXX
L$_s$-Nicotine
(Naturally occurring, levorotatory)



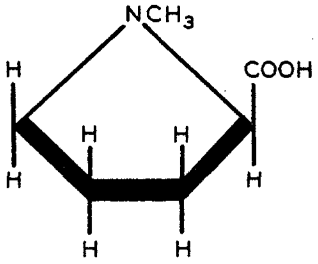
XXXI
D$_s$-Nicotine

instance is readily made through the close similarity of formulas XXVI for L$_s$-proline and XXX for natural nicotine. Natural nicotine appears to us to be best designated L$_s$-nicotine because it can be regarded as derivable, at least in principle, by the replacement of the COOH group of N-methyl-L$_s$-proline by a proper pyridine residue.

Levorotatory hygric acid has been correlated chemically with natural L$_s$-proline by Karrer and Widmer (10a); accordingly, the perspective formulas of the enantiomorphs of hygric acid are XXXII and XXXIII.



XXXII
L$_s$-Hygric Acid
(levorotatory)



XXXIII
D$_s$-Hygric Acid

It is important to observe that Emil Fischer's second convention is maintained in all the formulas XXII–XXXIII and that their derivation has included his first convention. The mechanical models that are specified by these stereo-formulas through his first and second conventions are entirely independent of all nomenclature, a matter that was emphasized by Fischer (11).

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