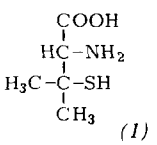
D-Penicillamine—Production and Properties

By Wolfgang M. Weigert, Heribert Offermanns, and Paul Scherberich[*]

The non-naturally occurring amino acid D-penicillamine, originally only of interest as a key substance in the structural elucidation and total synthesis of penicillins is gaining increasing importance as a therapeutic, particularly in the long-term treatment of rheumatoid arthritis. D-Penicillamine which previously was obtained semi-synthetically by degradation of penicillins can now be totally synthesized using a new process which starts from isobutyraldehyde, sulfur, ammonia, and hydrogen cyanide and yields the racemate which is then selectively resolved. The biochemical behavior of D-penicillamine—chelate formation with heavy metal ions, cleavage of disulfide bridges, and condensation with aldehyde groups—affords information related to its therapeutic activity.

1. Introduction

In 1943, during their investigations on the structural elucidation of penicillins, *Abraham et al.*[1, 2] ("The Oxford Group") found all penicillins to give a common degradation product which, in ignorance of its exact structure, was called "penicillamine" because of the presence of an amino group in the molecule. The β,β-dimethylcysteine structure *(1)* postulated by *Cornforth*[1] was later confirmed by the "Oxford Group" on investigation of its reactions and synthesis[1].



(1)

"Penicillamine" is levorotatory in alkine solution and, with respect to its absolute configuration, belongs to the D-series of amino acids. The correct chemical name for "penicillamine" is D-(−)-2-amino-3-mercapto-3-methylbutyric acid. Common synonyms are: D-(−)-β,β-dimethylcysteine; D-(−)-β-mercaptovaline; and D-(−)-2-amino-3-mercaptoisovaleric acid. In this report the substance will be called D-penicillamine.

D-Penicillamine *(1)* was at first solely of interest as a key substance in the structural elucidation of penicillins and as a central building block in their total synthesis[3, 4]. In 1956, D-penicillamine, which itself possesses no antibiotic activity, was first introduced into medicine as a chelating agent to accelerate the elimination of physiological heavy metals present in high, non-physiological concentrations (therapy of Wilson's disease) and non-physiological heavy metals (antidote in heavy metal poisoning)[5, 6].

D-Penicillamine is becoming increasingly important as a basis therapeutic in rheumatoid arthritis (chronic polyarthritis)[7–9]. Further new indications for D-penicillamine, *e. g.* chronic aggressive hepatitis[10–13] and multiple sclerosis[14], are becoming apparent.

Only pure D-penicillamine is of therapeutic importance as the racemate and the L-isomer are toxic. Further differences between D- and L-penicillamine lie in their antagonistic activity towards vitamin B_6 and their molecular biological behavior (see Section 4.3).

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2. Manufacture of D-Penicillamine

D-Penicillamine can be manufactured either semi-synthetically by degradation of penicillins or completely synthetically *via* the racemate. Production from penicillins has been considerably improved in the last few years[15]. A new process for the manufacture of fully-synthetic D-penicillamine *via* the racemate and its resolution ("Asinger process") is now also being carried out industrially. Both processes produce highly pure D-penicillamine which is then used as the active ingredient in drug manufacture[*].

2.1. Manufacture of D-Penicillamine by Degradation of Penicillins

On degradation of penicillins [*e. g.* penicillin G *(2)*, Scheme 1] both the β-lactam ring and the thiazolidine ring are opened. The β-lactam ring is best cleaved in the presence of alkali; the resulting penicilloic acid *(3)* is decarboxylated to penilloic acid *(4)*. As a 2-monosubstituted thiazolidine-4-carboxylic acid, *(4)* is relatively stable to hydrolysis. Thus cleavage to D-penicillamine *(1)* and the aldehyde *(5)* requires removal of one or both of the decomposition products from the hydrolysis equilibrium.

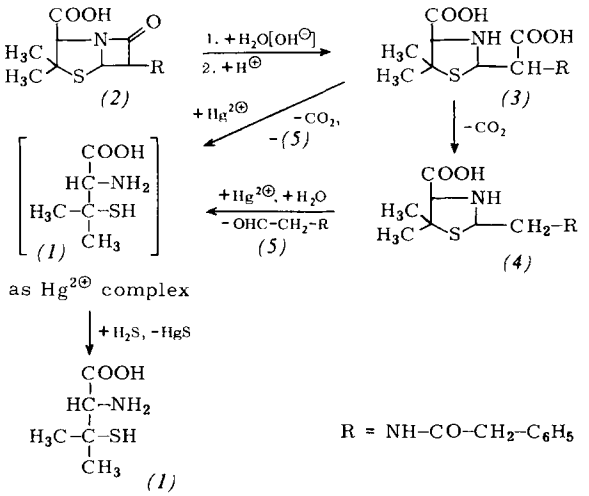
Preferably *(1)* is converted into the sparingly soluble 1:1 or 2:1-D-penicillamine-mercury(II) complexes and separated from the readily soluble product *(5)*[15, 16] (see Section 4.1 for the structure of the complexes).

Treatment with hydrogen sulfide liberates *(1)* from the mercury complex, mercuric sulfide being formed as by-product. Compound *(3)* can also be cleaved directly by mercuric salts. In this variant, decarboxylation and thiazolidine ring opening proceed simultaneously.

In another process the D-penicillamine-mercury 1:1 complex is kept in solution by addition of acid and the aldehyde *(5)*, after reaction with a carbonyl reagent, *e. g.* hydrazine or hydroxylamine, is separated by extraction with an inert organic solvent which is immiscible with water[17].

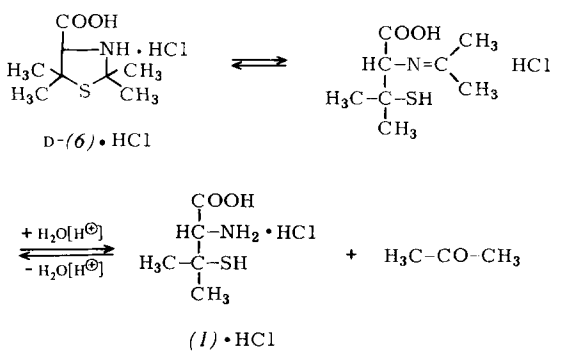
The use of other heavy metal salts in place of the expensive and toxic mercury salts has been recommended[18]. Without the use of heavy metal salts, *(3)* and *(4)* can be converted into D-penicillamine using 5,5-dimethyl-1,3-cyclohexanedione (dimedon)[19] or 4-hydroxycoumarin[20].

[*] D-Penicillamine (semisynthetic): Metalcaptase® (Knoll AG, Ludwigshafen/Heyl & Co, Berlin); D-penicillamine (fully synthetic): Trovolol® (Chemiewerk Homburg, Frankfurt (Main)/Bayer AG, Leverkusen).



Scheme 1

Purification of *(1)* is most suitably carried out by conversion into the hydrogen chloride of D-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid *(6)*. This latter product *(6)* is also known as "D-penicillamine-acetone adduct" or "isopropylidene-D-penicillamine" due to the ease with which it can be reconverted into acetone and *(1)*[30, 31].

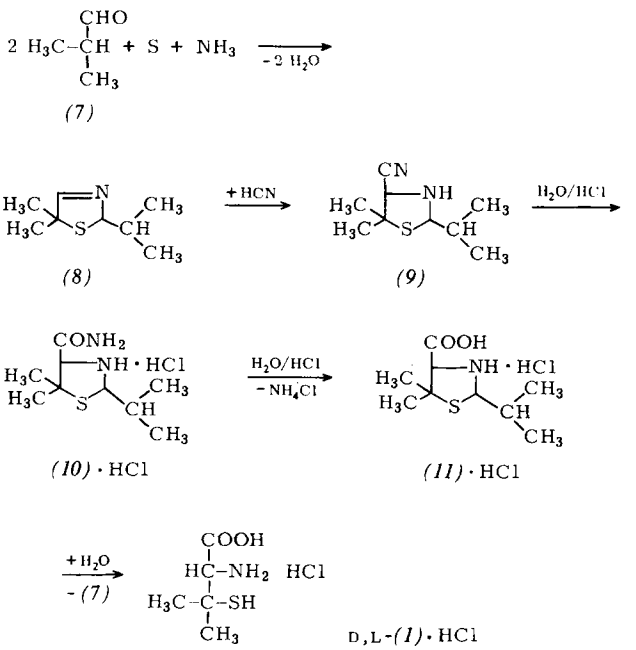


2.2. Manufacture of D-Penicillamine *via* the Racemate

The new fully synthetic process for the manufacture of D,L-penicillamine *via* 3-thiazoline starting from isobutyraldehyde, sulfur, ammonia, and hydrogen cyanide, and the new procedure for obtaining pure D-penicillamine from the racemate without the inevitable production of the L-isomer are based on the fundamental work of *Asinger et al.*[21–24] and have been developed by Degussa/Chemiewerk Homburg[*] to technical maturity[25]. Apart from this technique, which is an alternative to the hydrolysis of penicillins, there are several other procedures for the production of D,L-penicillamine and resolution of the racemate which were primarily developed in connection with the structural elucidation and total synthesis of penicillins during World War II. A description of these methods will be dispensed with here as a detailed review is already available[1]. However, we shall discuss two new synthetic routes, one of which starts from isocyanoethyl acetate and the other utilizes a modified Strecker synthesis.

2.2.1. Synthesis of D,L-Penicillamine *via* 3-Thiazoline

The simultaneous reaction of sulfur and ammonia with isobutyraldehyde *(7)* affords 2-isopropyl-5,5-dimethyl-3-thiazoline *(8)* in *ca.* 80% yield if the resulting water is azeotropically removed (see Scheme 2). Benzene, toluene, cyclohexane, chloroform, or preferably an excess of isobutyraldehyde itself can be used as the azeotropic partner[26]. The 3-thiazoline *(8)* itself is purified by rectification (b.p. 68—70°C/12 torr).



Scheme 2

Anhydrous hydrogen cyanide adds across the azomethine group of *(8)* to form 2-isopropyl-5,5-dimethylthiazolidine-4-carbonitrile *(9)* almost quantitatively. The hydrogen cyanide itself can be introduced either as a liquid or as a gas, or actually generated within the reaction mixture itself, for example from sodium cyanide and acids. If addition of HCN is carried out in an inert solvent, *e.g.* diethyl ether or petroleum ether, *(9)* can be isolated in crystalline form (m.p. ≈30°C) by cooling the reaction mixture. It is also possible to work in the absence of solvent and continue directly with the crude nitrile *(9)*.

In order to convert *(9)* into D,L-penicillamine the nitrile group must be saponified to a carboxyl group and the thiazolidine ring opened[27]. This is preferably carried out in several steps: *(9)* dissolved *e.g.* in methanol containing sufficient water to form the amide is treated with hydrogen chloride at 50—65°C. 2-Isopropyl-5,5-dimethylthiazolidine-4-carboxamide hydrogen chloride *(10)*·HCl is then obtained in crystalline form. *(10)*·HCl is subsequently hydrolyzed by refluxing with 10—20% hydrochloric acid to form 2-isopropyl-5,5-dimethylthiazolidine-4-carboxylic acid hydrogen chloride *(11)*·HCl[28]. Use of gaseous hydrogen chloride can be avoided by converting *(9)* into *(10)*·HCl by treatment with at least 30% aqueous hydrochloric acid at 40—70°C[29].

As a 2-monosubstituted thiazolidine derivative, *(11)*·HCl is extremely stable to hydrolytic ring opening[30, 31]. However it is possible to quantitatively convert *(11)*·HCl into D,L-penicillamine hydrogen chloride if the isobutyraldehyde *(7)* formed is azeotropically steam distilled out of the hydrolysis equilibrium[27]. In a variant of this procedure[32] the isobutyraldehyde is converted with a carbonyl reagent *(12)*, *e.g.* hydroxylamine, hydrazine, phenyl hydrazine, or semicarb-

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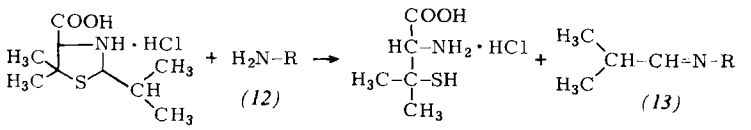
azide in aqueous solution into a derivative *(13)* which can be isolated by extraction with an inert organic solvent, *e.g.* toluene or chloroform, which is immiscible with water.



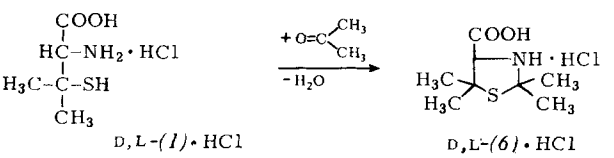
(11) · HCl D,L-*(1)* · HCl

R = OH, NH_2, NH–C_6H_5, NH–CO–NH_2

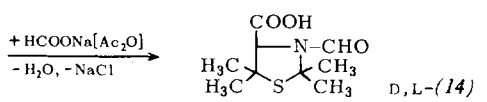
These methods for ring cleavage of *(11)* · HCl afford an aqueous solution of D,L-penicillamine hydrogen chloride containing ammonium chloride originating from saponification of the nitrile.

The D,L-penicillamine or its hydrogen chloride need not be isolated; after evaporation of the water it is preferable to react the D,L-penicillamine hydrogen chloride, with acetone in an organic solvent, *e. g.* toluene, which is immiscible with water. In this way crystalline D,L-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid hydrogen chloride, D,L-*(6)* · HCl is obtained in admixture with ammonium chloride. From this mixture D,L-penicillamine can be isolated in the pure form or converted into a suitable derivative for resolution.

A new procedure[33] using sodium formate in the presence of acetic anhydride in an organic solvent not miscible with water can be used to advantage for converting D,L-*(6)* · HCl into the corresponding *N*-formyl derivative D,L-*(14)* and permits removal of inorganic salts. The D,L-3-formyl-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid D,L-*(14)*, obtained in high yield and in excellent purity, is well suited for enantiomerization (see Section 2.2.3).



D,L-*(1)* · HCl D,L-*(6)* · HCl

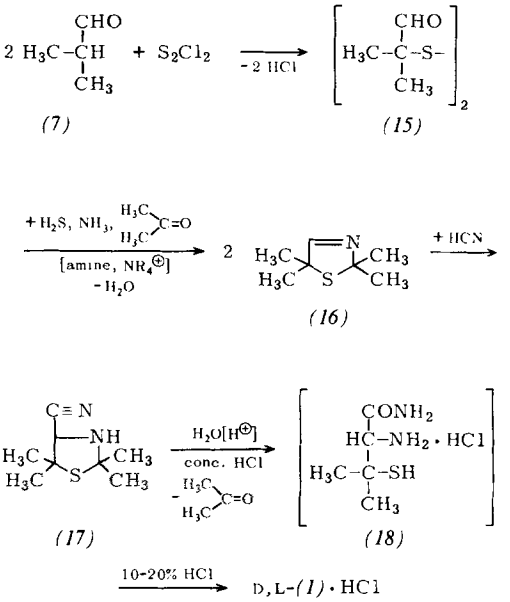


D,L-*(14)*

Using a variant of this technique, D,L-penicillamine can also be prepared from 2,2-dialkyl-5,5-dimethyl-3-thiazolines, and preferably from 2,2,5,5-tetramethyl-3-thiazoline *(16)* (Scheme 3). *(16)* cannot be obtained from isobutyraldehyde *(7)* in one step. For the preparation of *(16)*, isobutyraldehyde is initially reacted with disulfur dichloride to give 2,2'-dithiodiisobutyraldehyde *(15)*[34]. Simultaneous treatment of a mixture of *(15)* and acetone with hydrogen sulfide gas and ammonia gas furnishes *(16)*[35] in satisfactory yields provided that the reaction is carried out in the presence of amines and ammonium salts[36].

The nitrile *(17)* obtained by addition of hydrogen cyanide can be easily hydrolyzed to D,L-penicillamine hydrogen chloride without isolating the intermediate *(18)*[37].

This process does not require a steam distillation or use of a carbonyl reagent for ring cleavage since *(17)* is a 2,2-disubstituted thiazolidine derivative and thus undergoes facile hydrolytic cleavage[31]. Saponification of *(17)* to D,L-*(1)* · HCl has considerable advantages over the previously described pathway *via* the ester of tetramethylthiazolidine-4-carboxylic acid[21]. However, the ease of ring opening in *(17)* does not compensate for the disadvantage of the more complicated synthesis of the thiazoline *(16)* itself.

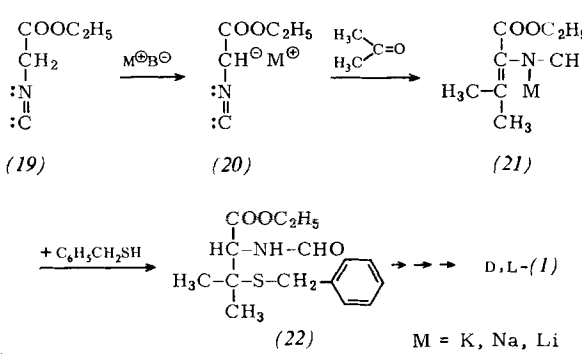


(7) *(15)* *(16)* *(17)* *(18)* D,L-*(1)* · HCl

Scheme 3

2.2.2. Synthesis of D,L-Penicillamine by Other Recent Methods

A general synthesis of *S*-benzyl-*N*-formylcysteines which is also suitable for the manufacture of D,L-*S*-benzyl-*N*-formylpenicillamine developed by *Schöllkopf* and *Hoppe*[38] starts from ethyl isocyanoacetate (see Scheme 4).



(19) *(20)* *(21)* *(22)* M = K, Na, Li

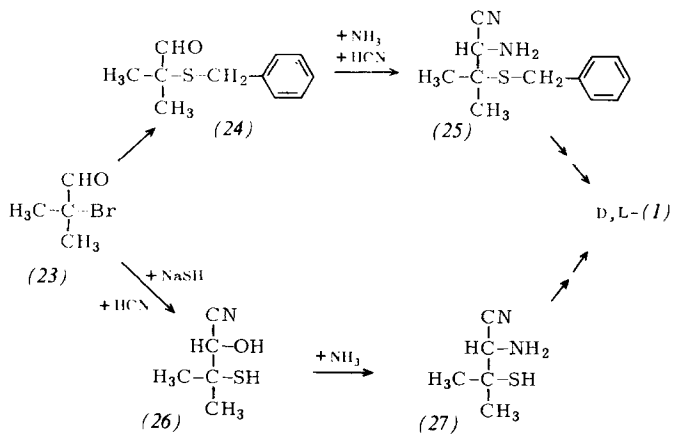
Scheme 4

Metalation of ethyl isocyanoacetate *(19)* by potassium *tert*-butoxide, sodium hydride, or *n*-butyllithium in tetrahydrofuran, and subsequent reaction of the metalated ester *(20)*

with acetone gives the metalated α-formylaminoacrylate *(21)* Treatment of *(21)* with thiobenzyl alcohol and hydrolysis with potassium hydroxide solution yields the ester *(22)* which can be selectively hydrolyzed to D,L-*S*-benzyl-*N*-formylpenicillamine or hydrolytically deformylated and then debenzylated to D,L-penicillamine.

D,L-Penicillamine can also be prepared by a kind of Strecker synthesis[39] (see Scheme 5) in which α-bromoisobutyraldehyde *(23)* is reacted with the sodium salt of thiobenzyl alcohol to give α-benzylthioisobutyraldehyde *(24)*. This product reacts with hydrogen cyanide and ammonia to form the nitrile *(25)*, which can be converted by hydrolysis and debenzylation into D,L-penicillamine in moderate yield.

According to *Ugi* and *Böttner*[40] α-hydroxy-β-mercaptoisovaleronitrile *(26)* can be obtained from α-bromoisobutyraldehyde by reaction with sodium hydrogen sulfide and hydrogen cyanide (see Scheme 5). *(26)* is converted by ammonia into *(27)*, which according to *Böttner*[41] can be saponified to D,L-penicillamine.



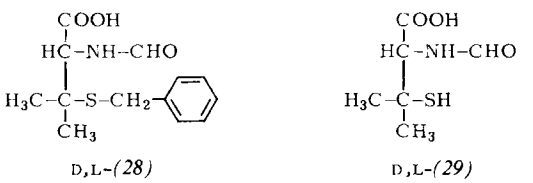
Scheme 5

The methods described in this section do not generally afford pure D,L-penicillamine but rather a derivative suitable for resolution.

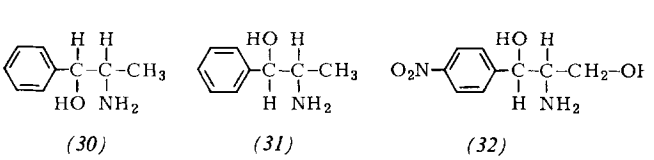
2.2.3. Isolation of D-Penicillamine from the Racemate

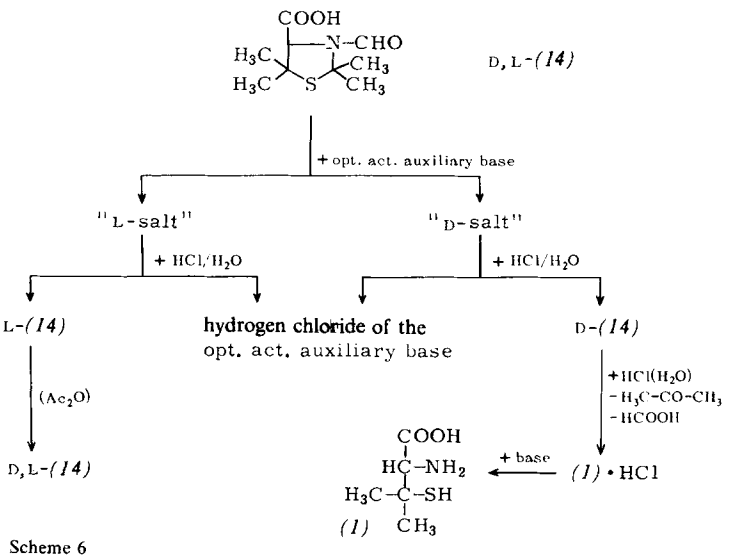
Owing to the widely differing biological properties of the optical antipodes of penicillamine (see Sections 1 and 4.3) it is necessary to quantitatively remove the undesirable L-isomer.

Optical resolution of D,L-penicillamine is performed by the classical method of transforming optical antipodes into diastereoisomers. Thus suitable derivatives of D,L-penicillamine are converted into the diastereomeric salts with alkaloids or other optically active auxiliary bases. Resolution is performed with *N*-acyl derivatives of D,L-penicillamine and of D,L-*S*-benzylpenicillamine as well as with the *N*-acyl derivatives of the condensation products of D,L-penicillamine with carbonyl compounds, especially D,L-3-formyl-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid, D,L-*(14)*. The first racemic resolution was achieved by the previously mentioned "Oxford Group"[42] who resolved D,L-*S*-benzyl-*N*-formylpenicillamine, D,L-*(28)* using the alkaloid brucine. D,L-*N*-Formylpenicillamine, D,L-*(29)* and D,L-*(14)* can also be enantiomerized with brucine[43, 44].



Other bases used in the racemic resolution of D,L-penicillamine are thebaine[45], quinidine[46], cinchonidine[46], (−)-ephedrine[44, 46], and (+)-pseudoephedrine[46]. Regarding the need to quantitatively remove the therapeutically undesirable L-penicillamine, the above optical bases are not wholly suitable for obtaining pure D-penicillamine because in some cases the salts of L-penicillamine derivatives crystallize from the solution first. This is a disadvantage since the diastereoisomer which crystallizes out first always possesses the higher purity[47, 48]. Moreover, the yields are unsatisfactory. For an economically viable separation of the antipodes the very expensive or highly toxic alkaloids can hardly be considered. L-





Scheme 6

Lysine[49], (−)-norephedrine *(30)*[50], (−)-pseudonorephedrine *(31)*[51], and D-(−)-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol *(32)*[52] are most suitable as auxiliary bases. These bases are preferentially reacted with D,L-*(14)* according to Scheme 6.

In all cases the amine salts of the D-penicillamine derivatives ("D-salts") are obtained as the more insoluble diastereoisomers. In suitable solvents the solubility differences between the diastereoisomeric salts are so large that quantitative removal of the L-penicillamine derivative becomes feasible. The "D-salts" are preferentially treated with dilute hydrochloric acid in the cold to form the water-soluble hydrogen chloride of the optical base and the sparingly water-soluble D-penicillamine derivative D-*(14)*. On deformylation and ring opening D-*(14)* furnishes pure D-penicillamine · HCl, which can be converted into free D-penicillamine *(1)* with, for example, triethylamine in an alcohol.

The amine salt of the L-penicillamine derivative ("L-salt") retained in the mother liquor on resolution is likewise cleaved with dilute hydrochloric acid to give L-3-formyl-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid L-*(14)*. This product is quantitatively racemized, for example, on refluxing in toluene containing catalytic quantities of acetic anhydride[53], and subjected to renewed resolution. It is thus possible—apart from the losses inevitably incurred by processing—to quantitatively obtain D-penicillamine from the racemic penicillamine derivatives. The optically active adjuvant bases can be almost completely recovered.

3. Physical Properties and Analysis of D-Penicillamine

D-Penicillamine *(1)* is a colorless crystalline powder with a weak odor typical of sulfur-containing amino acids, and a characteristic taste. It is relatively soluble in water but less so in alcohols and almost insoluble in ether, chloroform, carbon tetrachloride, and aliphatic or aromatic hydrocarbons.

(1) melts with decomposition at 202—206 °C (Mettler FP melting point apparatus, starting temperature 195 °C, heating rate 2°/min).

Qualitative identification of *(1)* is based on the reaction with iodine solution (decolorized due to oxidation of the SH group), ninhydrin (blue-violet color), phosphortungstic acid (deep blue color), and acetone (colorless precipitate)[54].

Quantitative determination is best carried out by titration against mercuric acetate solution in the presence of diphenylcarbazone as indicator[54]. Traces of impurities, *e. g.* D-penicillamine disulfide *(39)* may be detected either by TLC or, after silylation, by GLC.

In 1 N sodium hydroxide solution [5 g *(1)* in 100 ml] *(1)* is levorotatory. The pure compound *(1)* to be used as a drug constituent should have an optical rotation of −62.5 to −63.5° (at 20 °C in sodium D light = 589 nm). D-Penicillamine prepared according to the "Asinger process" is identical in its physical, spectroscopic, and chemical properties with the material obtained from penicillin. The stereochemical equivalence of the fully and semi-synthetic D-penicillamine can be confirmed by precision polarimetry and ORD spectroscopy. No traces of the L-isomer can be detected in either semi-synthetic or fully-synthetic D-penicillamine used in drugs formulation by present-day physical methods.

The optical purity can also be confirmed[56] by tests with *E. coli* bacteria, whose growth is selectively inhibited by L-penicillamine[55]. Again no difference was found between semi-synthetic and fully-synthetic D-penicillamine.

4. Biochemical Properties of D-Penicillamine

The biochemical properties of D-penicillamine are primarily based on three types of reaction in which the amino and mercapto groups are principally involved:

1) Chelate formation with heavy metal ions; 2) exchange reactions with low molecular and high molecular weight disulfides; 3) condensation with aldehydes.

4.1. Chelate Formation

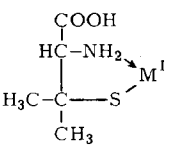
D-Penicillamine is a strong complexing agent and reacts with the majority of heavy metal ions, particularly with those with an affinity for S, to form chelates[57]. While D-penicillamine forms only a 1:1 complex of type *(33)*, *e. g.*, with $Cu^{\oplus}$ ions[58-61], it forms not only 1:1 complexes of types *(33)* and *(34)* but also 2:1 *(35)* or sometimes 3:1 complexes[59-63] with other heavy metal ions.

Depending on the number of functional groups involved in complex formation D-penicillamine functions as a bidentate *(33)* or tridentate ligand *(34)*.

Of the physiological metal ions which participate in complex formation with D-penicillamine, copper, zinc, iron, cobalt, manganese, and nickel deserve mention.

The therapeutic activity of D-penicillamine in the treatment of Wilson's disease is based upon chelate formation with protein-bound copper and its rapid excretion[59, 64, 65]. A similar mode of activity is assumed in for example the treatment of sclerodermia[66] and schizophrenia[67, 68] with D-penicillamine.

Chelate formation is also the basis for the antidote action of D-penicillamine in heavy metal poisoning, *e. g.* by mercury or lead compounds[69-71].



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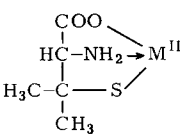

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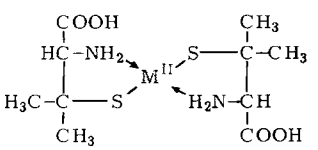


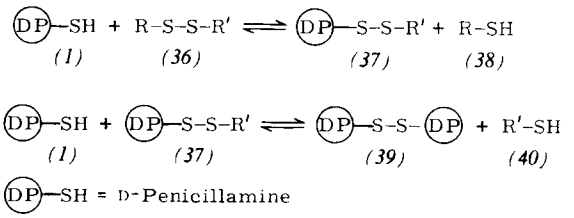
(35)

Undesirable side effects caused by elimination of zinc or other biometals can be avoided by substitution[72].

4.2. Exchange Reactions with Disulfides

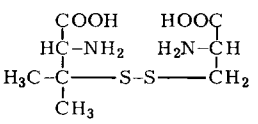
D-Penicillamine *(1)* can react with disulfides *(36)* in organisms according to Scheme 7[73-75]; the mixed disulfide of D-penicillamine *(37)* and the free thiol *(38)* are formed.

(37) can react further with D-penicillamine to give D-penicillamine disulfide *(38)* and the thiol *(40)*.



Scheme 7

The formation of the mixed disulfide *(41)* from D-penicillamine and L-cysteine and of the symmetrical disulfide *(39)* is decisive for the treatment of cystinuria and the associated formation of urinary calculus (cystine stones)[73, 76]. D-Penicillamine disulfide *(39)* and D-penicillamine L-cysteine disulfide *(41)* are much more soluble than cystine and are thus eliminated.

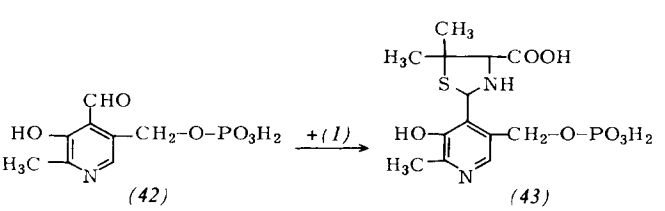


(41)

Similar exchange reactions can also take place with proteins linked intermolecularly through S—S bridges, *e. g.* immunoglobulins, pathological macroglobulins (rheumatic factors) which play an important role in the pathogenesis of rheumatoid arthritis[77–80]. It is suspected that the macroglobulins lose their pathogenic properties through depolymerization according to Scheme 7. This is partly the reason for the use of D-penicillamine in cases of rheumatoid arthritis. The depolymerizing activity of D-penicillamine on macroglobulins was confirmed by *in vitro* experiments[81, 82].

4.3. Reactions with Aldehydes

D-Penicillamine reacts with the aldehyde group of pyridoxal 5'-phosphate *(42)* to form the thiazolidine derivative *(43)*[83–85].

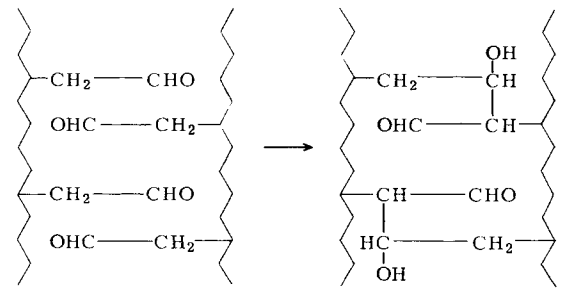


(42) *(43)*

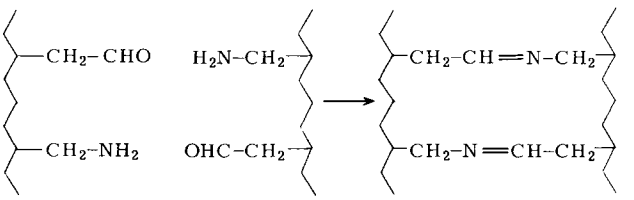
The co-enzyme *(42)* which is formed in the organism from vitamin B_6 plays an important role in amino acid metabolism, in the synthesis of coenzymes, as well as in the biosynthesis of hormones such as adrenalin.

Both isomers of penicillamine possess an anti-vitamin B_6 effect, that of the L-isomer being considerably stronger than that of *(1)*[86, 87]. For this reason, the therapy with D-penicillamine of high stereochemical purity is of particular importance. Vitamin B_6 deficiency symptoms caused by treatment with D-penicillamine are relatively rare and can be corrected by the simultaneous administration of vitamin B_6[88–90]. However, it appears that there is no direct correlation between the reaction of D-penicillamine with *(42)* and its therapeutic activity.

The crosslinking of collagen fibers (tropocollagens) soluble in physiological saline solution to insoluble precollagens plays an important roll in sclerodermia[91–93], chronic aggressive hepatitis[10], and rheumatoid arthritis[94]. The formation of insoluble precollagens is probably initiated by enzymatic oxidation of the ε-amino groups of lysine residues to aldehyde groups. It is assumed that the aldehyde groups are crosslinked *via* aldol condensations (Scheme 8) or by the formation of azomethine bridges (Scheme 9)[95–97].



Scheme 8



Scheme 9

D-Penicillamine reacts with the aldehyde groups of the soluble collagens forming thiazolidines and thus inhibits crosslinking[58, 97].

In contrast to other sulfur-containing amino acids, *e.g.* cysteine D-penicillamine is relatively stable in the organism, and thus its therapeutic activity is fully displayed. It is primarily converted by oxidation or exchange reactions according to Scheme 7 into the disulfides *(39)* and *(41)* and eliminated in this form[98]. Unlike L-penicillamine, D-penicillamine cannot be incorporated into proteins or inhibit protein synthesis[99–101].

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