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[Ph 75]

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Resolution of Optical Isomers by Thin-Layer Chromatography

Enantiomeric Purity of D-Penicillamine

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Penicillamine (**1**) is condensed with formaldehyde to form the enantiomeric 5,5-dimethylthiazolidinecarboxylic acids D-**3** and L-**3**. These enantiomers are separated by TLC on CHIRALPLATE®.

Dünnschichtchromatographische Enantiomerentrennung, Enantiomere Reinheit von D-Penicillamin

Es wird ein einfaches Verfahren zur Bestimmung der enantiomeren Reinheit von D-Penicillamin (D-**1**) beschrieben. Dazu wird **1** mit Formaldehyd zu **3** umgesetzt. Die Enantiomere von **3** lassen sich dc bei kurzen Analysezeiten trennen. Dabei kommt die mit einem optisch aktiven Selektor belegte Fertigplatte CHIRALPLATE® zum Einsatz.

Owing to its conceptual simplicity and manfested utility, the direct chromatographic separation of enantiomers upon chiral columns has been attempted many times. For most workers, the target has proven chimerical and, not withstanding the achievements of *Bayer*[1], *Blaschke*[2], *Cram*[3], *Gil-Av*[4], *König*[5], *Lochmüller*[6], *Pirkle*[7], and *Schurig*[8], there has been little portent development of broad spectrum chiral stationary phases of extended scope and utility. While no single chiral stationary

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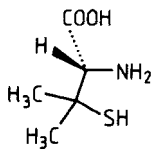
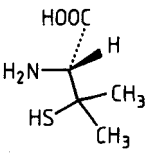
phase will ever suffice to separate all enantiomers, it is possible to rationally design chiral stationary phases that will separate the enantiomers of a wide assortment of products.

Having previously described a novel chiral stationary phase[9] for the liquid chromatographic resolution of racemic amino acid enantiomers, we focused our attention upon the analysis of racemates by *thin layer chromatography* (TLC). Today, gas[10] and high-performance liquid chromatography[11] are the methods of choice for the resolution of organic compounds in analysis. However, these methods necessitate the use of expensive apparatus and, in part, derivatization of the sample. A simple and rapid method – like TLC – of monitoring optical purity is therefore desirable.

Usually the optical rotation is used as an indication of the enantiomeric purity of commercial lots of the drug D-penicillamine (D-**1**). Estimation of the enantiomeric excess (e.e. value) from its optical rotation may give erroneous results. Optical rotations may be very sensitive to experimental conditions (e.g. purity of solvents, oxydation of penicillamine to its disulfide having a very high rotation value) and affected by unsuspected impurities. Thus, it is better to determine the enantiomeric purity by a direct method.



D-Penicillamine (D-**1**)
[(*S*)-configuration]

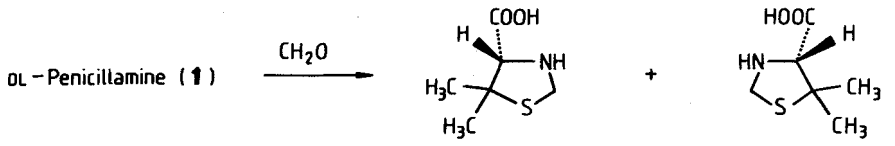


L-Penicillamine (L-**1**)
[(*R*)-configuration]
toxic

The enantiomeric purity plays an important role in determining the biological activity of molecules. Often small amounts of the minor enantiomer can change the activity of a compound[12]. The D-isomer of penicillamine (D-**1**) has been shown to be a potent drug in treating *Wilson's* Disease[13] and a wide variety of other illnesses[14–17]. On the other hand L-penicillamine (L-**1**) has been shown to be toxic[19–20]. In other words: penicillamine is a pharmacologically active asymmetric molecule whose D and L enantiomers possess different biological activities[21]. Accordingly, the resolution and quantification of penicillamine enantiomers has received a great deal of attention[22–26].

We now elaborate upon our report that amino acids can be resolved by TLC on a recently developed chiral stationary phase[27),28].

Our approach for the development of a simple direct enantiomeric TLC resolution of penicillamine (**1**) which can be used as an analytical probe of its enantiomeric purity involves a derivatization. Thus, **1** is condensed with formaldehyde to form the enantiomeric 5,5-dimethylthiazolidinecarboxylic acids D-**3** and L-**3**.



	D-3	L-3
Rf value	0.48	0.62

The TLC separation of the enantiomers is sensitive, fast, and very simple. Quantitative results may be obtained by densitometry.

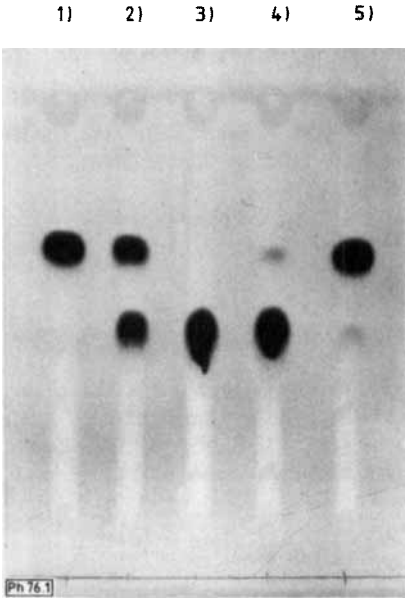


Fig. 1: Photograph of an original thin-layer chromatogram:
1) L-5,5-Dimethyl-4-thiazolidinecarboxylic acid (L-**3**)
2) D,L-5,5-Dimethyl-4-thiazolidinecarboxylic acid (D,L-**3**)
3) D-5,5-Dimethyl-4-thiazolidinecarboxylic acid (D-**3**)
4) 3 % L-5,5-Dimethyl-4-thiazolidinecarboxylic acid in D-5,5-Dimethyl-4-thiazolidinecarboxylic acid (D-**3**)
5) 3 % D-5,5-Dimethyl-4-thiazolidinecarboxylic acid in L-5,5-Dimethyl-4-thiazolidinecarboxylic acid (L-**3**)

Currently we are studying the application of TLC resolutions to additional compounds, especially those which have been resolved by ligand exchange high-performance liquid chromatography (HPLC).

Experimental Part

50 mg of penicillamine **(1)** was treated with 50 mg paraformaldehyde, 50 µl hydrochloric acid conc. and diluted with 2,5 ml isopropyl alcohol. After stirring for 2 h at 60°C the reaction mixture (2 µl) is directly applied to the TLC plate CHIRALPLATE®[28] and eluted with methanol/water/acetonitrile = 50 : 50 : 200 (vvv) about 30 min. After drying, the spots are visualized using 0.1 % ninhydrin reagent. The resolution of the enantiomers was so excellent that the respective antipodes could be determined at trace levels. The lowest level of detection was found to be ≥ 0.5 % with the conventional TLC technique.

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[Ph 76]

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Zur Stereochemie der 3-Oxo-5-phenyl-1-cyclopentancarbonsäuren, 10. Mitt.[1)]

Versuche zum Existenzbeweis der t-2-Methyl-3-oxo-r-5-(4-fluorphenyl)-t-1-cyclopentancarbonsäure

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Eingegangen am 26. April 1985

Beim Versuch, die *trans*-Cyclopentanon-carbonsäure **1** durch sauren Abbau des Triesters **6** zu erhalten, entsteht das 4-Methyl-1-(4-fluorphenyl)-cyclopenten-1-on-3 **(5)** in geringer Menge. Synthetisch wird **5** aus der *trans*-Cyclopentanoncarbonsäure **2** durch dehydratisierende Decarbonylierung gewonnen. **1** kann nicht nachgewiesen werden.

Stereochemistry of 3-Oxo-5-phenylcyclopentanecarboxylic Acids, X: Attempted Synthesis of t-2-Methyl-3-oxo-r-5-(4-fluorophenyl)-t-1-cyclopentanecarboxylic Acid

It is shown, that the *trans*-cyclopentanonecarboxylic acid **1** is not acessible by acidic degradation of the triester **6**. Instead, 4-methyl-1-(4-fluorophenyl)cyclopentene-1-one-3 **(5)** is obtained in low yield. By dehydrating decarbonylation, **5** can be prepared from the *trans*-cyclopentanonecarboxylic acid **2**.

Bisher waren Versuche fehlgeschlagen, die t-2-Methyl-3-oxo-*r*-5-(4-fluorphenyl)-*t*-1-cyclopentancarbonsäure **(1)** als vierte diastereomere Säure neben **2, 3** und **4** zu isolieren[2)]. Diese Beobachtung war bereits in der fluorfreien Verbindungsreihe gemacht worden[3)].

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