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The Action of Hydrazines upon Thiazolidine-4-carboxylic Acids I. Preparation of D-Penicillamine from D-Benzylpenilloic Acid*

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In the reaction of phenylhydrazine with D-benzylpenilloic acids (Ia or IIa) D-penicillamine was obtained in high yield and purity. The lack of reactivity of *N*-acyl derivatives (Ib or IIb) with phenylhydrazine indicated that the above reaction proceeded through the open chain imine tautomer of Ia or IIa (IV). The epimerisation of D-benzylpenilloic acid (Ia and IIa) in aprotic solvents was studied by using PMR spectroscopy.

D-penicillamine which was formerly used only as an antidote in heavy metal poisoning has been recently introduced into medicine as a therapeutic agent in rheumatoid arthritis, chronic aggressive hepatitis and multiple sclerosis**. These new applications have stimulated the interest in its simple and economical production.

D-penicillamine can be prepared either in a completely synthetic way or semisynthetically by the degradation of penicillins[1]. Since its racemic form and L-isomer are toxic and only the D-isomer is of therapeutic value, it is necessary to remove the toxic L-isomer quantitatively. As the classical methods for the separation of isomers[2] could hardly be considered economical, we paid more attention to the semisynthetic preparation of D-penicillamine from penicillins.

The preparation from penicillins was based on the treatment of 2-monosubstituted-5,5-dimethyl-thiazolidine-4-carboxylic acid with a heavy metal salt in water[3–6]. The formed D-penicillamine metal complex was decomposed by means of hydrogen sulfide, and the aldehyde (the main interfering byproduct) was removed by treating the solution with a carbonyl reagent to yield an insoluble derivative of aldehyde.

Though relatively stable to hydrolysis[7], 2-monosubstituted-5,5-dimethyl--thiazolidine-4-carboxylic acids are readily epimerized at *C*-2 upon heating in an aqueous solution, due to the opening of the thiazolidine ring.

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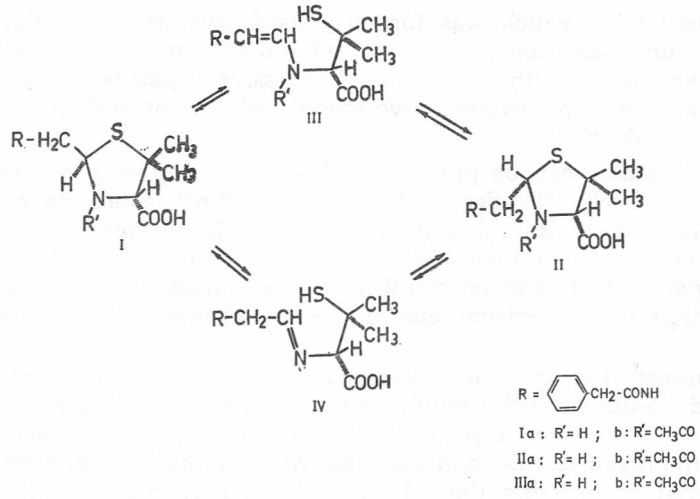
If a suitable carbonyl reagent is present, D-penicillamine can be isolated, since the aldehyde is removed from the solution in the form of the carbonyl derivative. Thus, in the recently reported preparations, only a carbonyl reagent, such as dimedone[8] or 4-hydroxycumarine[9], was used to precipitate aldehyde and to isolate D-penicillamine; metal salts were not used.

Certain disadvantages of these methods stimulated us to consider the possibility of producing D-penicillamine from corresponding penicillin derivatives, without the use of heavy metal salts, toxic hydrogen sulfide and expensive carbonyl reagents.

An extensive study of the epimerisation of 2-phenylacetamidomethyl-5,5--dimethyl-thiazolidine-4-carboxylic acid (D-benzylpenilloic acid) with the use of PMR spectroscopy has revealed that the opening of the thiazolidine ring takes place not only upon heating in an aqueous solution but also in aprotic solvents or in the melted state.

It is known that D-benzylpenilloic acid exists in two stereoisomers designated as D-alfa (monohydrate and anhydrous form) and D-beta (only anhydrous form)[10]. This assignment was given to distinguish the isomers in terms of their physical properties since the configurations at *C*-2 in thiazolidine were uncertain. A recent X-ray crystallografic analysis has shown that D-alfa--benzylpenilloic acid monohydrate posses the »*2R, 4S*« (Ia) configuration[11]. Evidently the D-beta isomer posses »2*S*, 4*S*« (IIa) configuration.



The PMR spectra of the D-alfa and D-beta isomers in DMSO-d_6 (Figure 1A and 1B) exhibited a difference essentially consisting only in a small difference in the chemical shifts of protons at *C*-4 (0.11 ppm) and the two methylene protons adjasent to *C*-2 in thiazolidine (0.29 ppm).

The PMR spectrum of »anhydrous« D-alfa-benzylpenilloic acid revealed that this form was in fact, a mixture of the D-alfa and D-beta isomers (Figure 1C).

The PMR spectra corroborated that the heating of the D-alfa or D-beta isomer in an alcohol solution resulted in epimerisation at *C*-2 in thiazolidine

and that the equilibrium mixture consisted of equal amounts of both isomers. Moreover, it was found that the same equilibrium mixture resulted when either of the isomers was heated in xylene, *N*,*N*-dimethylacetamide, dimethylsulfoxide or in the melt (Figure 1C).

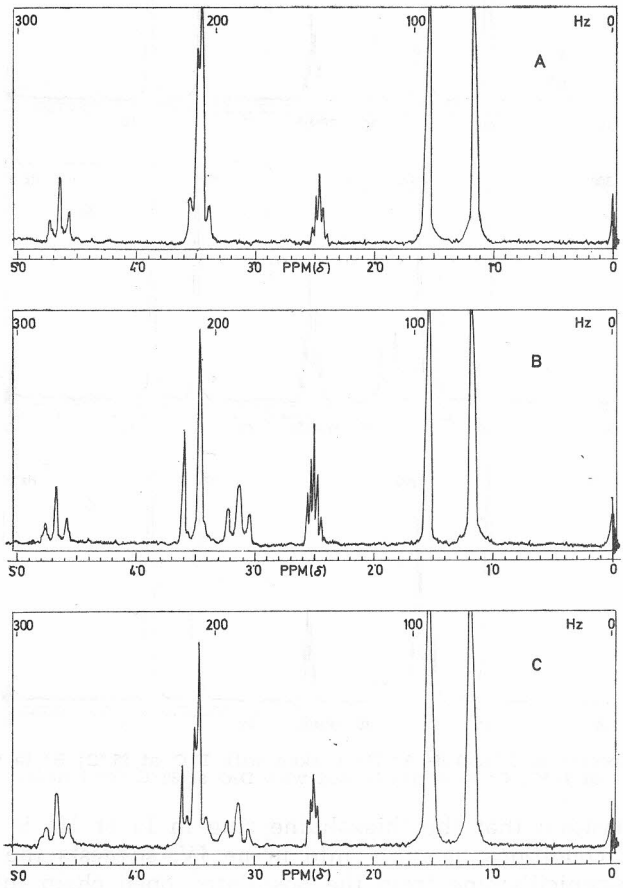


Figure 1. PMR spectra in DMSO-d_6 A: IIa; B: Ia; C: Ia or IIa subjected to epimerisation.

The PMR spectra also showed that neither of the isomers had undergone deuteration at the C-atom adjacent to C-2 in thiazolidine during epimerisation in the presence of deuterium oxide (Figure 2C). This is in contradiction with the assumption of Mozingo and Folkers that the epimerization occurs through intermediate III[12]. Several reports[13–15] indicate that in some derivatives thiazolidine exists in equilibrium with the open chain imine form. This is in favour of the idea that imine IV is one of the intermediates in the course of the epimerization of D-benzylpenilloic acids. The evidence that the *N*-acyl derivatives of both isomers (Ib or IIb) do not epimerize under the same reaction conditions as those for Ia or IIa, supports this assumption, since the *N*-acyl group would prevent the opening of the thiazolidine ring into the open chain imine IV.

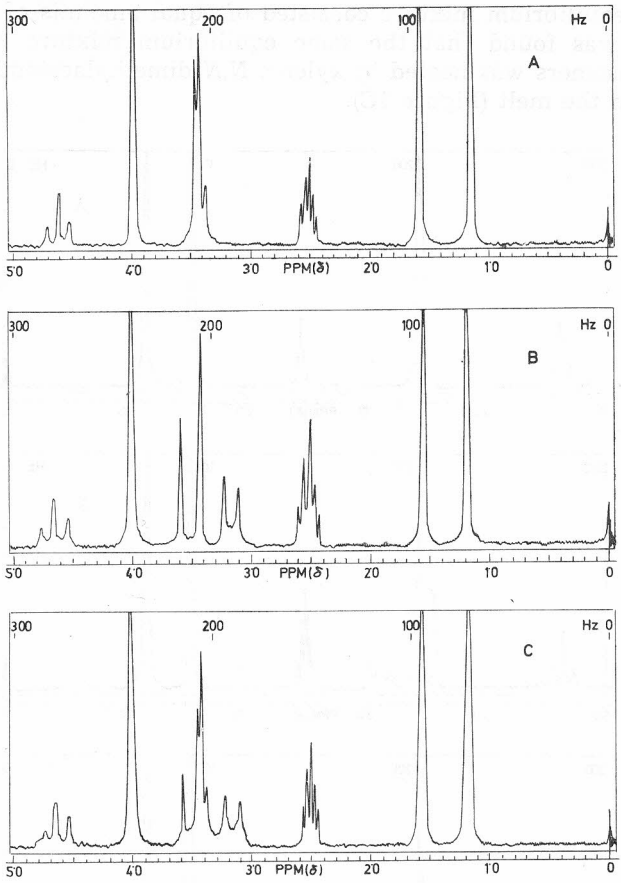
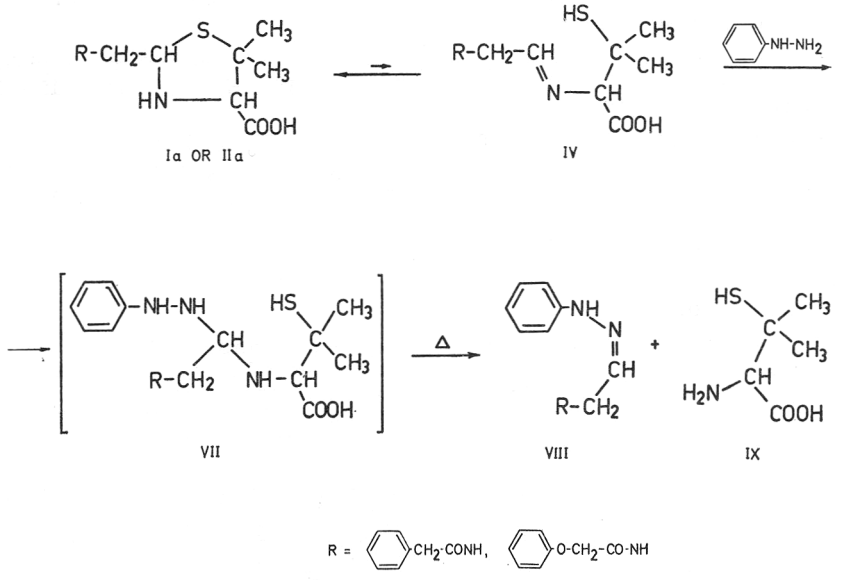


Figure 2. PMR spectra in DMSO-d_6 A: IIa shaken with D_2O at 25 °C; B: Ia shaken with D_2O at 25 °C; C: Ia or IIa heated with D_2O at 80 °C for 1 hour.

The observation that the thiazolidine ring in Ia or IIa is opened under nonhydrolytic conditions, possibly into imine IV, suggests the possibility of isolation of D-penicillamine from the postulated open chain intermediate by means of a suitable reagent. In several attempts with different reagents, the best results were obtained with the use of phenylhydrazine[16].

In the reaction of phenylhydrazine with D-benzylpenilloic acids (Ia or IIa) in organic solvents, D-penicillamine (IX) was formed within a short reaction time. Since, the phenylhydrazone of penilloaldehyde (VIII) was soluble in solvents, D-penicillamine crystallised from the solution in high yield and purity.

No direct evidence was obtained of the reaction of phenylhydrazine on the postulated open chain imine IV to yield reactive intermediate VII. However, this assumption was supported by the evidence that the *N*-acyl derivatives (Ib and IIb) did not react with phenylhydrazine under the same reaction conditions as those for Ia and IIa. In our opinion this observation suggests a nucleophilic addition of phenylhydrazine to imine IV rather than a direct nucleophilic attack on the C-2 in thiazolidine in Ia or IIa.



The method described for the preparation of D-penicillamine is certainly one of the most suitable and economical method. In this procedure one avoids the use of heavy metal salts and the use of expensive carbonyl reagents, as well as the complex procedure of isolation from a large volume of water solution.

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were recorded with a Model 257G Perkin-Elmer spectrometer. The uv spectra were recorded with an SP 800 Unicam spectrometer. The PMR measurements were done with an A-60 Varian in DMSO-d_6 with TMS as the internal standard. TLC was conducted on original plates (Merck, Kieselgel HF_{254}), followed by detection with iodine and water. Solvent systems, A: dichloromethane-methanol (4 : 1), B: *n*-butanol-acetic acid-water (4 : 1 : 1).

2R,4S-2-Phenylacetamidomethyl-5,5-dimethyl-thiazolidine-4-carboxylic acid monohydrate (D-alfa-Benzylpenilloic acid monohydrate) (Ia)

To a solution of potassium benzylpenicillin (3.72 g, 0.01 gmol) in water (20 ml), 25% solution of sodium hydroxide (1.7 ml) was added. The reaction mixture was stirred at 25 °C for 1 hour and acidified with 50% solution of sulphuric acid to pH 4. The solution was heated at 90 °C for 1 hour. At 75 °C, 50% solution of sulphuric acid was added to pH 2. After cooling, the crude precipitate was collected by filtration; yield 3.1 g (95%), m. p. 95—103 °C, one spot by TLC in solvent system B. The product was crystallized from water; m. p. 99—103 °C; $[\alpha]_D^{23} + 73.6^0$ (c 0.5, MeOH); 5.47% water (K. F.); Lit.[10]: m. p. 112—114 °C, $[\alpha]_D^{23} + 63.4^0$ (c 0.5, EtOH).

Ir spectrum: 3520(s), 3450(s), 3190(m), 3020(vs), 1680(vs), 1640(vs), 1600(vs), 1400(vs), 1340(vs) cm^{-1}

PMR spectrum δ: 1.18 (s, alfa-CH_3), 1.53 (s, beta-CH_3), 3.13 (t, $J = 7$, NCH_2), 3.42 (s, $PhCH_2$), 3.57 (s, C_4H), 4.66 (t, $J = 7$, C_2H), 7.25 (s, C_6H_5), 8.12 (t, $J = 6$, CONH).

2S,4S-2-Phenylacetamidomethyl-5,5-dimethyl-thiazolidine-4-carboxylic acid (D-beta-Benzylpenilloic acid) (IIa)

Ia (6.52 g, 0.02 gmol) was twice crystallized from methanol (25 ml) to yield 2.4 g (39%) of IIa, m. p. 162—164 °C, $[\alpha]_D^{23}$ + 35.7° (c 0.5, MeOH). Lit.[10]: m. p. 158—160 °C, $[\alpha]_D^{23}$ + 45° (c 0.45, EtOH).

Ir spectrum: 3310(m), 2970(s), 1655(vs), 1630(vs), 1535(vs), 1368(vs) cm^{-1}.

PMR spectrum δ: 1.17 (s, alfa-CH_3), 1.53 (s, beta-CH_3), 3.42 (t, J = 6, NCH_2), 3.42 (s, $PhCH_2$), 3.46 (s, C_4H), 4.65 (t, J = 5, C_2H), 7.25 (s, C_6H_5), 8.23 (t, J = 6, CONH).

2R,4S-2-Phenylacetamidomethyl-3-acetyl-5,5-dimethyl-thiazolidine-4-carboxylic acid hemihydrate (N-Acetyl-D-alfa-benzylpenilloic acid hemihydrate) (Ib)

To a solution of D-alfa-benzylpenilloic acid monohydrate (3.26 g, 0.01 gmol) and triethylamine (3.0 g, 0.03 gmol) in dichloromethane (50 ml), acetylchloride (1.57 g, 0.02 gmol) was added dropwise at 15 °C. The rection mixture was stirred at 20 °C for 3 hours. 0.1 M hydrochloric acid (40 ml) was added, the dichloromethane layer separated, dried over sodium sulphate and evaporated to dryness; yield 3.1 g (86%) Ib, m. p. 64—110 °C.

For analysis, the crude product was dissolved in dichloromethane (30 ml) and water (30 ml), and 10% solution of sodium hydroxide was added to pH 10. The water layer was separated, dichloromethane added (20 ml) and acidified with 10% solution of hydrochloric acid to pH 2. The dichloromethane extract was dried over sodium sulphate and evaporated to dryness (one spot by TLC in solvent system B), m. p. 74—110 °C (foam), $[\alpha]_D^{23}$ + 35.3° (c 0.5, MeOH), water 2.3% (K. F.).

Anal. $C_{17}H_{22}N_2O_4S \cdot 1/2\,H_2O$ (359.47) calc'd: C 56.80; H 6.45; N 7.79; S 8.92%
found: C 56.67; H 6.08; N 8.07; S 9.12%

Ir spectrum: 3300(m), 1730(s), 1635(b), 1540(s), 1387(vs), 1200(s), 728(m) cm^{-1}.

PMR spectrum δ: 1.33 (s, alfa-CH_3), 1.62 (s, beta-CH_3), 2.07 (d, J = 11, CH_3CO), 3.43 (s, $PhCH_2$), 3.55—4.18 (m, NCH_2), 4.27—4.72 (m, C_4H), 4.97—5.38 (m, C_2H), 3.27 (s, C_6H_5), 8.25 (b, CONH).

2S,4S-2-Phenylacetamidomethyl-3-acetyl-5,5-dimethyl-thiazolidine-4-carboxylic acid (N-Acetyl-D-beta-benzylpenilloic acid (IIb)

To a solution of IIa (3.08 g, 0.01 gmol) and triethylamine (2.02 g, 0.02 gmol) in dichloromethane (50 ml), acetylchloride (0.78 g, 0.01 gmol) was added dropwise at 15 °C. The reaction mixture was stirred at 15 °C for 2 hours. 0.1 M hydrochloric acid was added, the dichloromethane layer was separated, dried over sodium sulphate and evaporated to dryness. The solid was dissolved in methanol (40 ml), and water (40 ml) was added with stirring at 25 °C. Upon cooling to 0 °C, IIb crystallized from the solution and was collected by filtration; yield 2.72 g (78%) IIb, m. p. 180—184 °C (one spot by TLC in solvent system B).

For analysis, the crude product was twice crystallized from 50% methanol, m. p. 182—185 °C, $[\alpha]_D^{23}$ — 27.3° (c 0.5, MeOH). Lit.[17] m. p. 182—184 °C.

Anal. $C_{17}H_{22}N_2O_4S$ (350.43) calc'd: C 58.26; H 6.32; N 7.99; S 9.15%
found: C 58.51; H 6.40; N 8.07; S 9.00%

Ir spectrum: 3315(m), 1740(s), 1625(vs), 1545(s), 1380(s), 1200(s), 730(m) cm^{-1}.

PMR spectrum δ: 1.43 (s, alfa-CH_3), 1.43 (s, beta-CH_3), 2.09 (b, CH_3CO), 3.42 (s, $PhCH_2$), 3.50—3.85 (m, NCH_2), 4.62 (b, C_4H), 5.17—5.61 (m, C_2H), 8.83 (b, CONH).

Epimerisation of D-benzylpenilloic acids

1. a) IIa (50 mg) was dissolved in DMSO-d_6 (0.5 ml) at 25 °C in a PMR tube and the spectrum recorded (Figure 1A). After heating the tube at 80 °C for 1 hour, the PMR spectrum was recorded again at 25 °C (Figure 1C).

b) The same treatment was applied to Ia (Figure 1B and 1C).
c) According to the above procedure Ia or IIa treated in *N,N*-dimethylacetamide gave the same result.

2. a) IIa (0.25 g) was dissolved in methanol (5 ml) and the solution was refluxed for 30 minutes. The solution was evaporated to dryness under a reduced pressure and the residue dried at 20 °C/0.025 mm Hg for 1 hour. The dry residue (50 mg) was dissolved in DMSO-d_6 (0.5 ml) and the PMR spectrum recorded (Figure 1C).
b) The same treatment was applied to Ia (Figure 1C).
c) According to the above procedure, Ia or IIa treated in xylene gave the same result.
3. a) IIa (50 mg) was dissolved in DMSO-d_6 (0.5 ml) in PMR tube, D_2O (0.05 ml) was added, shaken for 30 minutes at 25 °C and the PMR spectrum recorded (Figure 2A). Additional D_2O (0.1 ml) was added and the tube heated at 80 °C for 1 hour. The PMR spectrum was recorded again at 25 °C (Figure 2C).
b) The same treatment was applied to Ia (Figure 2B and 2C).
4. Ia ($[\alpha]_D^{23}$ + 73.6°, water 5.47%) was dried at 70 °C/0.025 mm Hg for 24 hours and »anhydrous« Ia $[\alpha]_D^{23}$ + 59.5°, water 0.22%) was obtained. 50 mg was dissolved in DMSO-d_6 (0.5 ml) and the PMR spectrum recorded (Figure 1C).
5. a) IIa (60 mg) was melted at 160 °C, cooled, dissolved in DMSO-d_6 (0.5 ml) and the PMR spectrum was recorded at 25 °C (Figure 1C).
b) Ia (60 mg) was melted at 105 °C, cooled, dissolved in DMSO-d_6 (0.5 ml) and the PMR spectrum recorded at 25 °C (Figure 1C).
6. a) Ib (50 mg) was dissolved in DMSO-d_6 (0.5 ml) in a PMR tube and the spectrum recorded at 25 °C. An identical PMR spectrum was obtained when this sample was heated at 80 °C for 1 hour.
b) The same treatement was applied to IIb and the identical result was obtained.

Reaction of D-*penilloic acids with phenylhydrazine*

a) A mixture of D-beta-benzylpenilloic acid (20 g, 0.065 gmol) and phenylhydrazine (21 g, 0.195 gmol) in absolute xylene (100 ml) was heated under reflux for 1 hour. The D-penicillamine separated was filtered off. Yield 7.3 g (75%), m. p. 200—205 °C, $[\alpha]_D^{25}$ — —61° (c 5, 4% NaOH). The ir spectrum was superimposable on that of the authentic sample of D-penicillamine (Knoll AG).

The filtrate was allowed to stay at room temperature and 15 hours later the crystalline precipitate phenylhydrazone of phenylacetylaminoacetaldehyde was filtered off. Yield 12.3 g (71%), m. p. 131—134 °C. Recrystallization from benzene gave the analytical sample, m. p. 135—137 °C.

Anal. $C_{16}H_{17}N_3O$ (267.32) calc'd: C 71.88; H 6.41; N 15.72%
found: C 71.60; H 6.13; N 15.79%

Ir spectrum: 3400(s), 3265(vs), 1650(vs), 1625(vs), 1608(vs), 1514(vs) cm^{-1}.

Uv spectrum λ_{max}: 211 nm (log ε 4.869) and 257 nm (log ε 4.875).

PMR spectrum δ: 3.46 (s, $PhCH_2$), 3.88 (t, NCH_2), 6.51—7.46 (m, NC_6H_5, C_6H_5, N= =CH), 8.21 (t, CONH), 9.78 (s, PhNH, J = 5.5).

b) According to the above procedure, D-alfa-benzylpenilloic acid heated in benzene for 3 hours gave D-penicillamine in 70% and the corresponding hydrazone in 72% yield.

c) A mixture of D-phenoxymethylpenilloic acid (1 g, 0.003 gmol) and phenylhydrazine (0.5 g, 0.0046 gmol) heated under reflux in benzene for 1 hour, deposited D-penicillamine in 78% yield.

From the filtrate the solvent was removed by destillation and the dry residue recrystallized from ether. Phenoxyacetylaminoacetaldehyde hydrazone was obtained in 71% yield; m. p. 103—104 °C.

Anal. $C_{16}H_{17}N_3O_2$ (283.32) calc'd: C 67.82; H 6.05; N 11.29%
found: C 68.00; H 5.99; N 11.50%

Ir spectrum: 3310(s), 3280(s), 1650(vs), 1600(vs), 1220(vs), 1128(s), 1055(s), 750(vs), 690(vs) cm^{-1}.

Uv spectrum λ_{max}: 216 nm (log ε 3.857) and 237 nm (log ε 3.972).

PMR spectrum δ: 3.96 (t, NCH_2), 4.48 (s, OCH_2), 6.50—7.50 (m, OC_6H_5, NC_6H_5, N= =CH), 8.28 (b, CONH) and 9.77 (s, PhNH).

D-penicillamine prepared using procedure a), b) or c) was found to be identical.

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SAŽETAK

Reakcija hidrazina s tiazolidin-4-karbonskim kiselinama. I. Dobivanje D-penicilamina iz D-benzilpenilojeve kiseline

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Reakcijom fenilhidrazina i D-benzilpenilojeve kiseline (Ia ili IIa) dobiven je D-penicilamin u visokom iskorištenju i čistoći. Budući da *N*-acil derivati (Ib ili IIb) ne reagiraju s fenilhidrazinom u danim uvjetima, pretpostavlja se da je iminski tautomer od Ia ili IIa (IV) međuprodukt u toj reakciji. Studirana je epimerizacija D-benzilpenilojevih kiselina primjenom PMR spektroskopije.

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