Tetrahedron, 1962, Vol. 18, pp. 413 to 418. Pergamon Press, Ltd. Printed in Northern Ireland

THE SYNTHESIS OF α-METHYL-β-PHENYLISOCYSTEINE AND THE PREPARATION OF SUBSTITUTED THIAZOLIDINE-5-CARBOXYLIC ACIDS

G. STEFANOVIĆ, A. STOJILJKOVIĆ and M. STEFANOVIĆ
Institute of Chemistry, Faculty of Sciences, University of Belgrade, Yugoslavia

(*Received* 13 *May* 1961)

Abstract—(A) A new route of α-mercapto-β-amino-α,β-disubstituted carboxylic acids has been presented. By condensing 5-methyl-2-thiothiazolidine-4-one and benzylidene-N,N-bisacetamide, 5-methyl-5-[α-acetylaminobenzyl]-2-thiothiazolidine-4-one was obtained and this was converted through its oxime into the corresponding thiazolidine-2,4-dione. By the action of alkali the thiazolidine ring was cleaved and α-methyl-β-phenylisocysteine and its amide obtained.

(B) Substituted thiazolidine-5-carboxylic acids and the corresponding acid amides have been prepared by the condensation of α-methyl-β-phenylisocysteine and its amide with formaldehyde, benzaldehyde, ethyl α-formylcaprilate and ethyl α-formylphenylacetate. The acids obtained show no *in vitro* biological activity.

A. *α-Methyl-β-phenylisocysteine*

The syntheses of α-mercapto-β-amino acids are not numerous. The first synthesis of isocysteine was achieved by Gabriel[1] in 1905 by the decomposition of rhodaninedihydrouracil with concentrated hydrochloric acid. Schöberl and Braun[2] obtained isocysteine in 1935 from N-phthalyl-β-alanine, by the introduction of bromine at α-position and substitution by a mercapto group. In 1955 a patent[3] described the simultaneous preparation of cysteine and isocysteine from unsaturated nitriles. In the same year Knunyants *et al.*[4] succeeded in adding alkylsulphochlorides to the esters of α,β-unsaturated carboxylic acids obtaining α-alkylmercapto-β-amino acids and conversion into α-mercapto-β-amino acids (unusual addition of mercapto group to α-position).

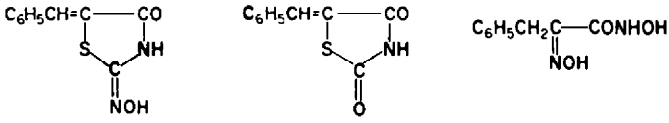
Rhodanine was selected as starting material for the synthesis of mercaptoamino acids since the cleavage of the rhodanine ring can afford the mercapto group and further, rhodanine contains an active methylene group which can be subjected to aminomethylation. Three ways for the introduction of an alkylamino group were attempted: (a) Mannich reaction, (b) reaction between hydroxylamine and benzylidenerhodanine and (c) the reactivity of arylidenebisacetamides with compounds containing an active methylene group.

(a) In an attempt to apply Mannich reaction to rhodanine, formaldehyde, dimethylamine and methylamine respectively, a mixture of di- and polyrhodanineaminomethane derivatives were obtained but no pure product could be isolated.

(b) It is known from Pozner's work[5] that the action of hydroxylamine on cinnamic acid produce β-aminocinnamic acid. Therefore, the action of hydroxylamine on

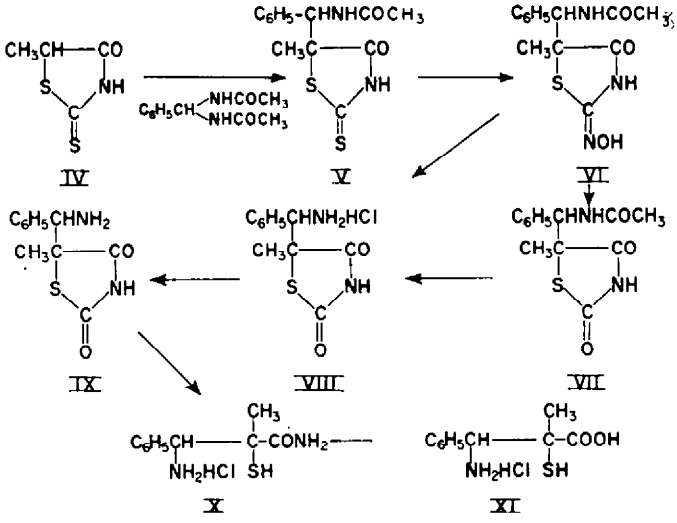
[1] S. Gabriel, *Ber. Dtsch. Chem. Ges.* **38**, 630 (1905).
[2] A. Schöberl and H. Braun, *Liebigs Ann.* **542**, 274 (1939).
[3] A. Ch. J. Opfermann, *Chem. Zentr.* 455 (1955); Oe.P. 177 766
[4] I. L. Knunyants, M. G. Linkova and P. G. Ignatenok, *Chem. Abstr.* **50**, 1592 (1956).
[5] Th. Pozner, *Liebigs Ann.* **389**, 1 (1912); *Ber. Dtsch. Chem. Ges.* **63**, 3078 (1930).

benzylidenerhodanine was tried and the oxime of benzylidenerhodanine (I) obtained ($C_6H_5CH{=}C-$ being unchanged). Acid hydrolysis of the oxime afforded 5-benzylidenethiazolidine-2,4-dione (II). This compound when treated with hydroxylamine gives a reaction mixture from which a new substance was isolated, characterized by analysis and colour reactions as the oxime of α-keto-β-phenylpropionhydroxamic acid (III).



I II III

The reactivity of arylidenebisamides with compounds containing an active methylene group[6] when applied to rhodanine should give acetylaminoalkylrhodanine and cleavage of the rhodanine ring the desired mercapto amino acid. However, it was found that benzylidenebisacetamide reacts with both hydrogen atoms of the active rhodanine group giving unsaturated compounds.[7] Therefore, it was necessary to use 5-methylrhodanine as starting material. The synthesis of α-methyl-β-phenylisocysteine was achieved by following reactions:



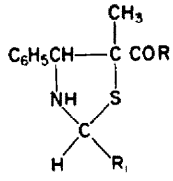
IV V VI VII VIII IX X XI

The condensation of 5-methylrhodanine (IV) and benzylidenebisacetamide in the presence of acetic acid anhydride gives 5-methyl-5-[α-acetylaminobenzyl]-2-thiothiazolidine-4-one in quantitative yield. Although the rhodanine ring is known to be readily cleaved by boiling with barium hydroxide,[8] we did not succeed in splitting the ring of V by the action of alkali. Therefore, V was treated with hydroxylamine and the oxime (VI) we converted to the corresponding thiazolidine-2,4-dione and the ring cleaved with alkali. When V is heated with hydroxylamine two products are obtained: oxime VI, m.p. 228° (isolated as monohydrate) and a compound VIa, m.p. 147° the

[6] Gj. Stefanović and J. Bojanović, *J. Org. Chem.* **17**, 816 (1952); Gj. Stefanović and M. Stefanović, *Ibid.* **21**, 161 (1956).
[7] Gj. Stefanović, M. Stefanović and M. Milanović, *Bull. Soc. Chim. Beograd* **20**, 313 (1955).
[8] J. Ginsburg and S. Bondzynski, *Ber. Dtsch. Chem. Ges.* **19**, 114 (1886).

structure of which is being investigated. Oxime VI when heated with concentrated hydrochloric acid gives: 5-methyl-5-[α-acetylaminobenzyl]-thiazolidine-2,4-diones (VII) and 5-methyl-5-aminobenzyl-thiazolidine-2,4-dione hydrochloride (VIII). Further treatment of VII with hydrochloric acid gives VIII. The yields of these two products depend upon the time of heating; since VIII is decomposed on prolonged heating it is desirable to isolate VII and then to remove its acetyl group. The free base, IX, isolated as monohydrate, may be obtained by treating an aqueous solution

TABLE I



Compound	R	R_1	M.p.
XII	—OH	H—	115°
XIII	—OH	C_6H_5—	202°
XIV	—OH	C_6H_5CH—$COOC_2H_5$—	101°
XV	—OH	$CH_3(CH_2)_5CH$—$COOC_2H_5$—	118°
XVI	—NH_2	C_6H_5—	175°
XVII	—NH_2	C_6H_5CH—$COOC_2H_5$—	95°
XVIII	—NH_2	$CH_3(CH_2)_5CH$—$COOC_2H_5$—	126°

of VIII with sodium bicarbonate. Cleavage of the thiazolidine ring of the free base with 10 per cent potassium hydroxide yields α-methyl-β-phenylisocysteineamide (X) as the hydrochloride; treatment with hydrochloric acid affords α-methyl-β-phenylisocisteine hydrochloride (XI). Compounds X and XI give a violet colour with ferric chloride in ethanol solution and a red colour with sodium nitroprusside in alkaline solution.

α-Methyl-β-phenylisocysteine and its amide may possess X-ray protecting activity in agreement with compounds of similar structure.[9]

B. *Substituted thiazolidine-5-carboxylic acids*

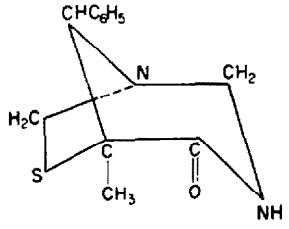
Cysteine and its derivatives are known to condense with carbonyl compounds producing substituted thiazolidine-4-carboxylic acids. With the discovery that penicillin contains a thiazolidine moiety and that its hydrolysis offers 5,5-dimethylthiazolidine-4-carboxylic acid many of these acids have been synthesized and their biological activities examined.[10,11]

As thiazolidine-5-carboxylic acids have not been reported, the condensation of α-methyl-β-phenylisocysteine with several carbonyl compounds has been carried out.

α-Methyl-β-phenylisocysteine and its amide have been condensed with formaldehyde, benzaldehyde, ethyl α-formylcaprilate and ethyl α-formylphenylacetate and the new compounds are listed in Table 1.

[9] E. Felder, F. Bonati and S. Bianch. *Experientia* **15**, 32 (1959).
[10] L. K. Werner, A. Wettstein and K. Miesher, *Helv. Chim. Acta* **30**, 432 (1947).
[11] H. Soloway, F. Kipnis, J. Ornfelt and P. Spoerri, *J. Org. Chem.* **70**, 1667 (1947); I. Schmolka and P. Spoerri, *J. Chem. Soc.* **22**, 943 (1959).

The condensation of α-methyl-β-phenylisocysteineamide with formaldehyde produces a compound of molecular formula $C_{12}H_{14}N_2OS$; this indicating that not only the cyclization of thiazolidine ring but the formation of a methylene bridge between the acid amide group and the imine group of thiazolidine ring takes place. The skeleton of the compound probably corresponds to 6-thia-1,3-diazobicyclo-(3,2,1)-octane (XIX), a bicyclic system of thiazolidine and pyrimidine.



Substituted thiazolidine-5-carboxylic acids listed in Table 1, in form of soluble sodium salts show no biological activity *in vitro*.

EXPERIMENTAL

Melting points are not corrected.

Alcoholic solutions of hydroxylamine were prepared as follows: Sodium (1 mole) was slowly added to absolute ethanol (500 ml). Then a solution of hydroxylamine hydrochloride (1 mole) in minimal quantity of water was added (10 ml of water dissolves 14 g of hydrochloride), the reaction mixture cooled and sodium chloride filtered off.

Oxime of 5-benzylidenerhodanine (I). The mixture of benzylidenerhodanine (30 g) and alcoholic hydroxylamine [prepared from sodium (6·2 g), absolute ethanol (200 ml) and hydroxylamine hydrochloride (18·7)] was refluxed for 100 min. After standing overnight at room temp the product was filtered off (19·5 g), but concentration of the filtrate yielded an additional 2·5 g giving a yield of 22 g (73%). The product is insoluble in water and ether but crystallizes from hot ethanol, m.p. 220° (decomp). (Found: C, 55·01; H, 3·84; N, 12·98; S, 14·68; $C_{10}H_8O_2N_2S$ requires: C, 54·52; H, 3·63; N, 12·73; S, 14·57%).

5-Benzylidenethiazolidine-2,4-dione (II). The oxime I (13 g) was refluxed with conc hydrochloric acid (350 ml) for 4 hr in oil bath at 150°. On cooling the precipitate (8·5 g) was washed with water and recrystallized from ethanol, m.p. 242°. An additional amount was obtained on evaporation of the solvent, giving a total yield of 75%. (Found: C, 58·82; H, 3·57; N, 7·07; S, 15·15; $C_{10}H_7O_2NS$ requires: C, 58·53; H, 3·41; N, 6·82, S, 15·61%).

Oxime of α-keto-β-phenylpropionhydroxamic acid (III). Compound II was refluxed for 3 hr with an alcoholic solution hydroxylamine [prepared from sodium (3·4 g), absolute ethanol (200 ml) and hydroxylamine hydrochloride (10·3 g)]. By evaporation of the solvent *in vacuo* 2 g were obtained. The product does not contain sulphur, is slightly soluble in ethanol, readily soluble in hot water from which it crystallizes on long standing, m.p. 197°; positive colour test with ferric chloride and copper sulphate. (Found: C, 55·83; H, 5·29; N, 14·47; $C_9H_{10}O_3N_2$ requires: C, 55·66; H, 5·15; N, 14·43%).

5-Methyl-5-[α-acetylaminobenzyl]-2-thiothiazolidine-4-one (V). A mixture of 5-methylrhodanine[12] (73·5 g; 0·5 mole), benzylidenebisacetamide (103 g; 0·5 mole) and acetic acid anhydride (102 g; 1 mole) was heated on an oil bath till dissolved and the heating continued for 2 hr at 150°. The cooled reaction mixture was treated several times (5–6) with icy water to remove the excess of acetic anhydride. The solidified product was dissolved in cold 10% sodium hydroxyde (2–2·51) and the alkaline solution slowly acidified with cold 10% hydrochloric acid yielding 140 g (93%); m.p. 70–140°. The product could not be recrystallized from common solvents. (Found: C, 52·15; H, 4·82; N, 8·67; S, 21·73; $C_{13}H_{14}O_3N_2S_2$ requires: C, 53·03; H, 4·79; N, 9·51; S, 21·78%).

Oxime of 5-methyl-5-[α-acetylaminobenzyl]-thiazolidine-2,4-dione (VI). Compound V (140 g; 0·05 mole) was boiled with alcoholic solution of hydroxylamine [prepared from sodium (23 g),

[12] B. Holmberg, *J. Prakt. Chem.* **81**, 455 (1923).

alcohol (600 ml) and hydroxylamine hydrochloride (69·5)] till the evolution of hydrogen sulphide ceased (6 hr). The reaction mixture was left overnight in a refrigerator and the product (80 g; 53%), m.p. 218–224°, recrystallized from ethanol and dried (2 hr *in vacuo*) at 90°, m.p. 228°. (Found: C, 53·48; H, 5·09; N, 14·29; S, 11·14; $C_{13}H_{15}O_3N_3S$ requires: C, 53·23; H, 5·15; N, 14·33; S, 10·93 %). Monohydrate: (Found: C, 50·30; H, 5·41; N, 13·39; S, 10·55; $C_{13}H_{15}O_3N_3S{\cdot}H_2O$ requires: C, 50·15; H, 5·50; N, 13·50; S, 10·30%). $\%H_2O$ (Found: 5·87; requires 5·78%). The filtrate was concentrated to ½ volume and left in refrigerator (48 hr). The precipitate obtained was filtered off and recrystallized from ethanol m.p. 153–160° (VIa). Further evaporation afforded an additional amount of VIa.

5-Methyl-5-α-acetylaminobenzylthiazolidine-2,4-dione (VII) *and 5-methyl-5-α-aminobenzylthiazolidine-2,4-dione* (IX)

(a) The oxime VI (10 g) was boiled with conc HCl for 30 min. The cold reaction mixture was diluted with water and the precipitate (5 g) recrystallized from ethanol, m.p. 253°. The product (VII) is insoluble in benzene and water, soluble in hot ethanol and sodium hydroxide. (Found: C, 56·16; H, 4·94; N, 9·79; S, 11·37; $C_{13}H_{14}O_3N_2S$ requires: C, 56·10; H, 5·07; N, 10·06; S, 11·51%). The filtrate was made slightly alkaline and the product (3 g) recrystallized from ethanol, m.p. 125°. It is soluble in sodium hydroxide and mineral acids, insoluble in benzene and ether. (Found: C, 56·02; H, 5·06; N, 11·99; $C_{11}H_{12}O_3N_2S$ requires: C, 55·91; H, 5·21; N, 11·85; S, 13·75%) Monohydrate: (Found: C, 51·85; H, 5·37; N, 10·99; S, 12·70; $C_{11}H_{12}O_3N_2S{\cdot}H_2O$ requires: C, 51·95; H, 5·55; N, 11·02; S, 12·62%). $\%H_2O$ (Found: 7·48 after drying at 55° in vacuum for 2 hr; requires: 7·0%). The pure hydrochloride was isolated by dissolving IX in dilute HCl, evaporating the solvent *in vacuo* and by recrystallization from absolute ethanol. m.p. 202° (Found: C, 48·81; H, 5·14; N, 10·19; $C_{11}H_{12}O_3N_2S{\cdot}HCl$ requires: C, 48·43; H, 4·80; N, 10·27%.

(b) Oxime VI (80 g) was refluxed with conc HCl (300 ml) for 3 hr. The reaction mixture was diluted with water (700 ml), neutralized with NaOH and made slightly alkaline with $NaHCO_3$ and the product (40 g) m.p. 119–122° collected. It can be used without further purification.

α-Methyl-β-phenylisocysteineamide hydrochloride (X). Compound IX was refluxed (8 hr) with 10% aqueous KOH, the cooled reaction mixture neutralized to litmus and the unsaponified product filtered off (5 g). The filtrate was acidified to congo red (evolution of CO_2) and evaporated to dryness *in vacuo* at 50°. The residue was extracted twice with absolute ethanol (1 l.). The extracts were evaporated to ½ volume and left in refrigerator (48 hr). The product (11 g) was filtered off and further evaporation of filtrate yielded an additional amount (7 g). It recrystallized from ethanol (with a drop of HCl), m.p. 190°. (Found: C, 48·50; H, 6·16; N, 10·09; $C_{10}H_{14}ON_2S{\cdot}HCl$ requires: C, 48·68; H, 6·08; N, 11·36%).

α-Methyl-β-phenylisocysteine hydrochloric XI. Compound X (5 g) was boiled with conc HCl for 30 min and the reaction mixture left in refrigerator to cool (24 hr). The precipitate (4 g) recrystallized from ethanol (some drops of HCl added), m.p. 205°. It crystallizes with one mole of water, is readily soluble in water, hot ethanol, insoluble in ether and gives positive ferric chloride and nitroprusside tests. (Found: C, 45·24; H, 6·08; N, 5·14; $C_{10}H_{13}O_2NS{\cdot}HCl{\cdot}H_2O$ requires: C, 45·19; H, 6·06; N, 5·27%).

2,4-Diphenyl-5-methylthiazolidine-5-carboxylic acid XIII. To a solution of XI (1·3 g) in absolute ethanol (10 ml), benzaldehyde (0·69 g) and anhydrous sodium acetate (0·4 g) were added. The mixture was heated to boiling and then left at room temp for 2 hr. The product (1·6 g) obtained after the addition of water (50 ml) was recrystallized from ethanol, m.p. 202°. It is soluble in hot ethanol and $NaHCO_3$, less soluble in ether and benzene, insoluble in water. (Found: C, 68·02; H, 5·93; N, 4·82; $C_{17}H_{17}O_2NS$ requires: C, 68·20; H, 5·72; N, 4·68%).

2-Phenylcarbethoxymethyl-4-phenyl-5-methylthiazolidine-5-carboxylic acid (XIV). The mixed solutions of XI (1·3 g) in water (10 ml) and ethyl α-formylphenylacetate (1 g) in ethanol (70 ml) were left to evaporate in a vacuum desiccator over P_2O_5 and solid KOH. The residue was washed first with petroleum ether–ether and then dissolved in a small amount of ethanol, precipitated by addition of water (0·6 g) and recrystallized from a mixture of ether and petroleum ether, m.p. 101°. (Found: C, 65·18; H, 6·16; N, 4·22; $C_{21}H_{23}O_4NS$ requires: C, 65·44; H, 6·01; N, 3·63%).

2-Hexylcarbethoxymethyl-4-phenyl-5-methylthiazolidine-5-carboxylic acid XV. The mixed solutions of XI (1·3 g) in water (10 ml) and ethyl α-formylcaprilate (1 g) in ethanol (10 ml) were left to evaporate in a vacuum desiccator over P_2O_5 and solid KOH. The residue was washed with petroleum

ether, filtered, washed with water (0·8 g) and recrystallized from 70% ethanol, m.p. 118°. The product is readily soluble in ether and benzene, insoluble in water. (Found: C, 63·73; H, 7·74; N, 3·76; $C_{21}H_{23}NO_2S$ requires: C, 64·09; H, 7·94; N, 3·56%).

2,4-*Diphenyl-5-methylthiazolidine-5-carboxylic acid amide* XVI. To a solution of X (1·2 g) in absolute ethanol (10 ml) freshly distilled benzaldehyde (0·6 g) and anhydrous sodium acetate (0·5 g) were added. The reaction mixture was refluxed for 10 min, until all the amide had reacted (negative ferric chloride test). After cooling the mixture was poured into water (50 ml) and the precipitate (1·5 g) recrystallized from ethanol, m.p. 174–175°. (Found: C, 68·74; H, 6·21; N, 9·13; $C_{17}H_{18}ON_2S$ requires: C, 68·42; H, 6·08; N, 9·39%).

2-*Phenylcarbethoxymethyl-4-phenyl-5-methylthiazolidine-5-carboxylic acid amide*, XVIII. Ethyl α-formylphenylacetate (0·8 g) was added to a solution of X (1·2 g) in water (5 ml). The mixture was left in a vacuum desiccator over P_2O_5 and solid KOH for 5 days. The product was treated with petroleum ether and (1 g) recrystallized from a mixture of petroleum ether and ether m.p. 95°. (Found: C, 65·43; H, 5·80; $C_{21}H_{24}O_3N_2S$ requires: C, 65·60; H, 6·29; N, 7·28%).

2-*Hexylcarbethoxymethyl-4-phenyl-5-methylthiazolidine-5-carboxylic acid amide*, XVIII. Ethyl α-formylcaprilate (1·1 g) and X (1·2 g) were dissolved in water (10 ml) and the reaction mixture left in a vacuum desiccator over P_2O_5 and KOH for 7 days. The product was treated with a mixture of petroleum ether and ether (3:1), filtered, washed with water (1·3 g) and recrystallized from 70% ethanol, m.p. 126°. It is insoluble in water and petroleum ether but readily soluble in alcohol and ether. (Found: C, 64·26; H, 8·10; N, 6·98; $C_{21}H_{32}O_3N_2S$ requires: C, 64·25; H, 8·22; N, 7·21%).

Compound XIX. α-Methyl-β-phenylisocysteine amide hydrochloride (X; 1 g) was dissolved by heating in water (10 ml) and formaldehyde (1 ml) was added to the warm solution. The reaction mixture was left to cool and the product (1 g) recrystallized from methanol, m.p. 195° (Found: C, 61·40; H, 6·10; N, 11·72; C, 61·36; H, 6·12; N, 11·85; $C_{12}H_{14}N_2OS$ requires: C, 61·54; H, 5·99; N, 11·96%).