107. *The Conversion of Sucrose into Thiazole Derivatives. Part I. Sulphanilamidothiazoles Derived from Lævulic Acid.*

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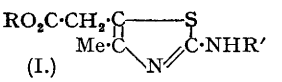
Lævulic acid has been converted into 2-*sulphanilamido*-4-*methylthiazole*-5-*acetic acid* and its *ethyl* ester. The former has the greater *in vitro* antibacterial activity and forms very soluble sodium and calcium salts. A number of basic salts of certain thiazole derivatives are described.

It is well established that sulphathiazole and some of its derivatives are among the most successful of the sulphanilamide drugs in use today. However, sulphathiazole has the disadvantage of low solubility in water; its *N*-acetyl derivative is still less soluble, and it is the latter compound which, formed during the detoxication process in the body, is the cause of the renal disorders which sometimes follow sulphathiazole therapy.

Sulphanilamidothiazoles and *N*-acetylsulphanilamidothiazoles, which are more soluble in water, are of special interest, and two are now described. Sulphanilamidothiazoles which do possess this increased solubility have now been prepared from sucrose through lævulic acid as the essential intermediate.

Ethyl β-bromolævulate (Conrad and Guthzeit, *Ber.*, 1884, **17**, 2285) by condensation with thiourea furnishes ethyl 2-amino-4-methylthiazole-5-acetate (I; R = Et, R′ = H) and hydrolysis of this compound with barium hydroxide solution gave the acid (I; R = R′ = H) which alternatively is obtained by condensation of β-bromolævulic acid with thiourea. Attempts to prepare 2-N^4-acetylsulphanilamido-4-methylthiazole-5-acetic acid from it by condensation with *N*-acetylsulphanilyl chloride were unsuccessful, indicating the zwitterionic character of the thiazole amino-acid. The condensation, however, proceeded smoothly when carried out with the ester (I; R = Et, R′ = H) and gave *ethyl* 2-N^4-*acetylsulphanilamido*-4-*methylthiazole*-5-*acetate*. This on hydrolysis with dilute hydrochloric acid gave 2-*sulphanilamido*-4-*methylthiazole*-5-*acetic acid hydrochloride*, from which the free *base* (I; R = H, R′ = *p*-$NH_2 \cdot C_6H_4 \cdot SO_2$) was obtained by warming it with water. The solubility of this was 0·113 g./100 c.c. of solution as compared with 0·04 g./100 c.c. of solution for sulphathiazole itself. Moreover, the new compound formed sodium and calcium salts which were very soluble in water.



(I.)

Ethyl 2-sulphanilamido-4-methylthiazole-5-acetate (I; R = Et, R′ = *p*-$NH_2 \cdot C_6H_4 \cdot SO_2$) was eventually obtained by condensation of ethyl 2-amino-4-methylthiazole-5-acetate with *p*-nitrobenzenesulphonyl chloride to give *ethyl* 2-p-*nitrobenzenesulphonamido*-4-*methylthiazole*-5-*acetate*, which, by catalytic hydrogenation over Raney nickel, was converted into (I; R = Et, R′ = *p*-$NH_2 \cdot C_6H_4 \cdot SO_2$). This could, by acetylation, be converted into the *N*-acetyl sulphanilamide derivative originally obtained by condensation of *N*-acetylsulphanilyl chloride with ethyl 2-amino-4-methylthiazole-5-acetate.

Compound.	Blood media.	Hartley's broth media.					
	Streptococcus hæmolyticus.	*Streptococcus hæmolyticus.*	*Escherichia coli.*	*Proteus vulgaria.*	*Pseudomonas aeroginosa.*	*Streptococcus aureus.*	*Clostridium welchii.*
Ethyl 2-*p*-nitrobenzenesulphonamido-4-methylthiazole-5-acetate	10	7	>50	—	—	—	—
Ethyl 2-sulphanilamido-4-methylthiazole-5-acetate	5	10	5	>100	>100	>100	>100
2-Sulphanilamido-4-methylthiazole-5-acetic acid	2	5	50	>100	>100	>100	30
Sulphathiazole	0·3	15	5	10	5	>100	5

Figures refer to mg. of material per 100 c.c. necessary to stop the growth of the organism under standardised conditions.

Results of some interest were obtained when the salts of these thiazole derivatives were prepared. In general, monohydrochlorides were produced, even in the case of ethyl 2-amino-4-methylthiazole-5-acetate, 2-amino-4-methylthiazole-5-acetic acid and ethyl 2-sulphanilamido-4-methylthiazole 5-acetate, in all of which there are two potentially basic centres. Since both

ethyl 2-N^4-acetylsulphanilamido-4-methylthiazole-5-acetate and ethyl 2-*p*-nitrobenzenesulphonamido-4-methylthiazole-5-acetate form salts, it is probable that it is the nitrogen atom in the ring which is involved in salt formation. The behaviour of 2-sulphanilamido-4-methylthiazole-5-acetic acid is anomalous in so far as it forms a dihydrochloride even though this is not stable and loses hydrogen chloride on keeping. It would have been more readily explicable had ethyl 2-sulphanilamido-4-methylthiazole-5-acetate formed a mono-salt, since then the difference might have been attributable to the zwitterion effect.

2-Sulphanilamido-4-methylthiazole-5-acetic acid and its ethyl ester and also ethyl 2-(*p*-nitrobenzenesulphonamido)-4-methylthiazole-5-acetate have been tested *in vitro* for their bacteriostatic action, and the results are shown on p. 590 in comparison with sulphathiazole itself.

These results show that 2-sulphanilamido-4-methylthiazole-5-acetic acid shows some promise as a chemotherapeutic agent since its bacteriostatic activity is quite appreciable and its solubility properties show such advantages over sulphathiazole. It is of interest to note that ethyl 2-*p*-nitrobenzenesulphonamido-4-methylthiazole-5-acetate, although it does not possess the sulphanilamido-grouping as such, does show some activity.

Experimental

Ethyl 2-Amino-4-methylthiazole-5-acetate.—This was prepared by the method of Conrad and Schmidt (*Annalen*, 1895, **285**, 203) using a slight excess of thiourea (4·0 g.) in water (30 c.c.) and ethyl *β*-bromolævulate (11·15 g.) in alcohol (20 c.c.). It was obtained in nearly colourless needles on recrystallisation from 80% aqueous alcohol, m. p. 123° (4·06 g.; 40·6%). The *hydrochloride* was obtained by passing dry hydrogen chloride into a solution of the base in sodium-dried ethyl alcohol at 0° until saturation was complete. Ether was then added, and the crystals which separated recrystallised from ethyl alcohol–ether in shining, colourless plates, m. p. 150° (Found: C, 40·7; H, 5·0; N, 11·7. $C_8H_{12}O_2N_2S$,HCl requires C, 40·60; H, 5·5; N, 11·9%).

2-*Amino-4-methylthiazole-5-acetic Acid.*—(*a*) The above ester (1·0 g.) was heated over a gauze for 3 hours with twice the calculated amount of barium hydroxide in water (75 c.c.). The mixture was then cooled and 5N-sulphuric acid added carefully until all the barium had been precipitated. Some slight decomposition of the thiazole derivative occurred during neutralisation as indicated by the evolution of hydrogen sulphide. The barium sulphate was filtered off and the aqueous solution concentrated under reduced pressure to small bulk. On cooling, crystals separated which, recrystallised from water in needles, had m. p. 274—275°, and were identical with the 2-amino-4-methylthiazole-5-acetic acid obtained below (0·55 g.; 63·9%).

(*b*) The acid was prepared according to the method of Conrad and Schmidt (*loc. cit.*) from thiourea (4·0 g.; 1·1 moles) and *β*-bromolævulic acid (10 g.). Recrystallisation from water gave the acid (5·0 g.; 56·7% of theory), m. p. 275° (Conrad and Schmidt give m. p. 259—260°) (Found: C, 42·1; H, 5·0; N, 16·2. Calc. for $C_6H_8N_2SO_2$: C, 41·9; H, 4·7; N, 16·3%). The *hydrochloride* was obtained by heating the acid with 5N-hydrochloric acid for a few minutes. On cooling, crystals separated which were recrystallised from water containing a little dilute hydrochloric acid; m. p. 228° (Found: C, 34·4; H, 4·6; N, 13·6. $C_6H_8N_2O_2S$,HCl requires C, 34·5; H, 4·4; N, 13·4%).

Ethyl 2-N^4-*Acetylsulphanilamido-4-methylthiazole-5-acetate.*—To ethyl 2-amino-4-methylthiazole-5-acetate (4·0 g.) in dry pyridine (40 c.c.) was added *N*-acetylsulphanilyl chloride (5·16 g., 1·1 moles) in dry pyridine (16 c.c.) at 0°. After 24 hours at room temperature, the reaction mixture was poured into ice-cold water and acidified with dilute hydrochloric acid at 0° (Congo-red). The *product* gradually separated during several hours as a fine crystalline powder. It recrystallised from aqueous alcohol in fine cream crystals, m. p. 203—204° (5·65 g.; 71·14%) (Found: C, 48·6; H, 4·7; N, 10·6. $C_{16}H_{19}O_5N_3S_2$ requires C, 48·4; H, 4·8; N, 10·6%). An early attempt to purify the crude product by suspending it in water, adding 5N-ammonia until the solution was alkaline to thymol blue, and refluxing the resulting reddish-brown solution with decolorising charcoal for ¾ hour, gave a straw-coloured solution which, when cooled to 0° and acidified with dilute hydrochloric acid, yielded crystals of 2-N^4-*acetylsulphanilamido-4-methylthiazole-5-acetic acid* (Found: C, 45·3; H, 4·5; N, 11·1. $C_{14}H_{15}O_5N_3S_2$ requires C, 45·5; H, 4·1; N, 11·4%); this, after recrystallisation from alcohol, had m. p. 228° and depressed the m. p. of the ethyl ester.

Ethyl 2-N^4-*Acetylsulphanilamido-4-methylthiazole-5-acetate hydrochloride* was prepared by dissolving the free base in sodium-dried ethyl alcohol and saturating the solution with dry hydrogen chloride at 0°. The salt, which separated on addition of ether, crystallised from ethyl alcohol–ether in colourless crystals, m. p. 206—207° (Found: C, 43·8; H, 5·0. $C_{16}H_{19}O_5N_3S_2$,HCl requires C, 44·3; H, 4·7%).

Hydrolysis of Ethyl 2-N^4-*Acetylsulphanilamido-4-methylthiazole-5-acetate.*—(a) *Using* 2N-*hydrochloric acid.* The acetyl derivative (5·0 g.) was suspended in 2N-hydrochloric acid (50 c.c.) and dissolved by heating on a water-bath for 1 hour. On cooling, fine colourless needles separated. These were collected and recrystallised from N-hydrochloric acid, giving long colourless needles of *2-sulphanilamido-4-methylthiazole-5-acetic acid dihydrochloride*, m. p. 235—236° (decomp.). Yield, 4·57 g., increased to 4·87 g. (theoretical) by working up the mother liquors (Found: C, 36·4; H, 3·9; N, 10·7. $C_{12}H_{13}O_4N_3S_2$,2HCl requires C, 36·0; H, 3·8; N, 10·5%). Under anhydrous conditions the dihydrochloride slowly decomposed and after 3 months it analysed as a *monohydrochloride* (Found: C, 39·0; H, 4·17; Cl, 10·5. $C_{12}H_{13}O_4N_3S_2$,HCl requires C, 39·6; H, 3·9; Cl, 9·8%).

(b) *Using* 10% *sodium hydroxide.* The acetyl derivative (1·0 g.) in 10% sodium hydroxide (10 c.c.) was heated on a water-bath for 2 hours, allowed to cool, and treated with excess of 5N-hydrochloric acid. The crystals which separated were recrystallised from N-hydrochloric acid and were identical with the product obtained in (a); m. p. 235—236° (0·80 g.; 83·4%).

Reacetylation of 2-Sulphanilamido-4-methylthiazole-5-acetic Acid Hydrochloride.—To the amine hydrochloride (0·1 g.), suspended in dilute acetic acid (5 c.c.), was added acetic anhydride (5 c.c.), and the mixture shaken until a clear solution was obtained. After a few minutes crystals separated, which were collected and recrystallised from ethyl alcohol; m. p. alone or in admixture with the 2-N^4-acetylsulphanilamido-4-methylthiazole-5-acetic acid previously described, 228°.

2-Sulphanilamido-4-methylthiazole-5-acetic Acid.—The hydrochloride (m. p. 235—236°; 1·5 g.) was suspended in water (50 c.c.) and dissolved by refluxing on a wire gauze for 1 hour. The crystals which separated on cooling were recrystallised from water giving colourless flakes of *2-sulphanilamido-4-methylthiazole-5-acetic acid*, m. p. 246—247° (1·1 g.; 86·3%) (Found : C, 43·9; H, 3·8; N, 12·5; NH_2 by nitrite titration, 4·88. $C_{12}H_{13}O_4N_3S_2$ requires C, 44·0; H, 4·0; N, 12·8; NH_2, 4·9%).

Ethyl 2-p-*Nitrobenzenesulphonamido-4-methylthiazole-5-acetate.*—*p*-Nitrobenzenesulphonyl chloride (2·5 g.; 1·1 moles) dissolved in dry pyridine was slowly added at 0° to ethyl 2-amino-4-methylthiazole-5-acetate (2·0 g.) also in dry pyridine (20 c.c.). After 12 hours at room temperature the reddish-brown solution was poured into ice-cold water and acidified to Congo-red at 0° with dilute hydrochloric acid. The crude product which separated recrystallised from acetone in long, yellow needles of the *ester*, m. p. 186—187° (2·8 g.; 94·9%) (Found : C, 43·9; H, 3·9; N, 11·4. $C_{14}H_{15}O_6N_3S_3$ requires C, 43·6; H, 3·9; N, 10·90%). The *hydrochloride* was prepared by dissolving the ester (0·05 g.) in dry methyl alcohol, and saturating the solution with dry hydrogen chloride at 0°. On slow evaporation of the solution in a vacuum desiccator, crystals separated and were recrystallised from anhydrous methyl alcohol containing dry hydrogen chloride; m. p. 236° (Found : C, 40·1; H, 3·9. $C_{14}H_{15}O_6N_3S_2$,HCl requires C, 39·9; H, 3·8%).

Ethyl 2-Sulphanilamido-4-methylthiazole-5-acetate.—2-*p*-Nitrobenzenesulphonamido-4-methylthiazole-5-acetate (1·0 g.) was dissolved in methyl alcohol (150 c.c.) and hydrogenated over Raney nickel at room temperature. After filtration and evaporation of the solvent, the crude product was recrystallised from acetone–water; needles of ethyl 2-sulphanilamido-4-methylthiazole-5-acetate containing acetone of crystallisation were thereby obtained, m. p. 184° (0·7 g.; 75·9%). After several days at room temperature the crystals had changed in form with conversion into the anhydrous *compound*, m. p. 137°; solubility in water was found to be 0·04725 g./100 c.c. solution (Found : C, 47·3; H, 4·9; N, 11·8; NH_2 by nitrite titration, 4·51. $C_{14}H_{17}O_4N_3S_2$ requires C, 47·34; H, 4·8; N, 11·8; NH_2, 4·51%).

The *monohydrochloride* was obtained by dissolving the free base in sodium-dried ethyl alcohol and saturating the solution with dry hydrogen chloride at 0°. The solution was then evaporated to dryness in a vacuum desiccator and the crude salt recrystallised from ethyl alcohol–ether to give fine colourless flakes, m. p. 228° (Found : C, 42·4; H, 4·7; N, 10·9. $C_{14}H_{17}O_4N_3S_2$,HCl requires C, 42·8; H, 4·6; N, 10·7%).

Acetylation of Ethyl 2-Sulphanilamido-4-methylthiazole-5-acetate.—Acetic anhydride (5 c.c.) was added to the free base (0·1 g.) suspended in 5 c.c. of dilute acetic acid. After being shaken until a clear solution was obtained the mixture was kept for 24 hours at room temperature and then poured into ice-cold water. The product slowly separated on standing, and when recrystallised from aqueous alcohol was identical with the ethyl 2-N^4-acetylsulphanilamido-4-methylthiazole-5-acetate, m. p. 203—204°, obtained previously.

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