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SYNTHESIS OF A DEGRADATION PRODUCT OF CEPHALOSPORIN C AND RELATED SULFUR-CONTAINING α-TETRONIC ACIDS

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In the course of their elegant elucidation of the structure of Cephalosporin C, Abraham and Newton[1] obtained two sulfur-containing lactones, to which the α-tetronic acid structures I and II were assigned on the basis of their chemical and spectral properties. Because of the significance of the structure of these lactones for the total structural argument and of the relevance of α-tetronic acids of this type for the chemistry of the cephalosporins in general, we wish to describe an unambiguous synthesis of I and of related substances.

β-Dimethylaminomethyl-α-tetronic acid hydrochloride, readily obtainable[2] from pyruvic acid, dimethylamine hydrochloride and formaldehyde, was treated in cold aqueous solution with silver carbonate to give quantitatively the zwitterion III: m.p. 115-118° (dec.),[3] $\lambda_{max}^{H_2O}$ 267 mμ (ε = 9000), λ_{max}^{KBr} 5.80, 6.15, 7.60, 8.95, 9.80, 10.60, 12.75 μ, found: C, 53.46; H, 7.11; N, 8.96. Treatment of III with one mole of potassium thioacetate in DMF for 15 minutes at 75° under nitrogen and evaporation of the mixture in high vacuum resulted in an almost quantitative yield of the dipotassium salt of I

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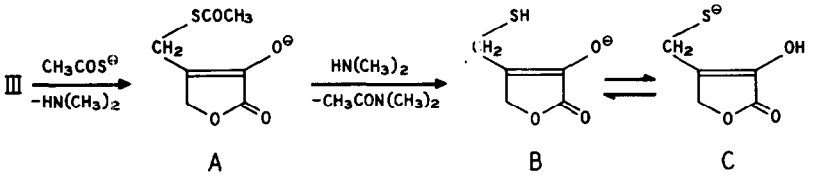
1 E. P. Abraham and G. G. F. Newton, Biochem. J. 79, 377 (1961).

2 C. Mannich and M. Bauroth, Ber. 57, 1108 (1924).

3 All melting points in capillaries. NMR spectra in $CDCl_3$ solutions on Varian A-60 instrument.

together with N,N-dimethylacetamide (detected by vapor phase chromatography) and hydrogen sulfide. On stirring the dipotassium salt with aqueous N HCl, the free dienol I crystallized in an overall yield of 84%.[4] One recrystallization from methanol furnished the pure compound: m.p. 147-148°, λ_{max}^{EtOH} 236 mμ (ε= 13,700), $\lambda_{max}^{0.01N\ NaOH}$ 281 mμ (ε= 13,600), λ_{max}^{KBr} 3.03, 5.75, 5.90, 8.77 and 12,83 μ. Mobility toward the anode in pH 7.0 buffer (collidine acetate, 0.05 M in acetate, 14 V/cm, 3 hours): 4.9 cm. Found: C, 46.42; H, 4.02; S, 12.31. The above constants as well as those for the dimethyl ether prepared with diazomethane: m.p. 74.5-75°, λ_{max}^{EtOH} 229 mμ (ε= 19,750), λ_{max}^{KBr} 5.65, 6.00, 6.89, 7.88-7.93, 8.80, 9.67, 9.93, 11.42, 12.88 and 13.15 μ. NMR: 6 protons at 6.02τ (methyl groups), 4 at 6.50τ (bridge methylenes), 4 at 5.22τ (ring methylenes),[5] found: C, 50.06; H, 4.72; S, 11.00; OCH_3, 21.96, are in good agreement with published data for the degradation product from *Cephalosporin C*. *Identity was kindly confirmed by Dr. E. P.* Abraham by direct comparison of the two products.

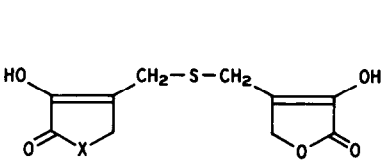


4 The sulfide I could also be prepared, albeit in lower yield, by using sodium hydrosulfide in place of potassium thioacetate or by using tetramethylenesulfone as the solvent. In aqueous systems, reverse Mannich reaction rather than replacement of the dimethylamino group takes place.

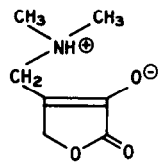
5 Long range coupling of J≅1 c.p.s. between ring and bridge methylene protons is a characteristic feature of all spectra.

The formation of I is visualized as proceeding from III via the thioacetyl enolate A, which is aminolyzed by the liberated dimethylamine to yield B and dimethylacetamide. The anion B is in equilibrium with C and attack of the latter on B results in the dienolate of I with loss of H_2S.

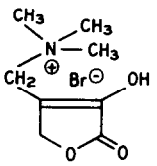
To prepare the β-mercaptomethyl-α-tetronic acid VII and its disulfide VI, it was necessary to avoid the formation of the anions B and C by aminolysis of the enolate A. This was achieved by first converting the



I X = O
II X = S



III



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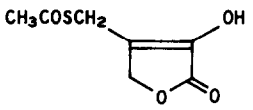

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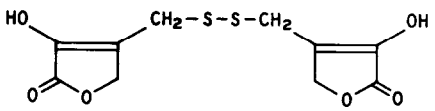

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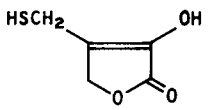
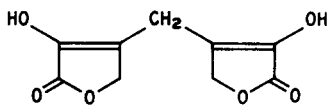


VII



VIII

zwitterion III into the unstable quaternary salt IV[6] by treating an acetone suspension with ten moles of methyl bromide for 18 hours at 5° (found: $Br^{\ominus}$, 31.6%) followed by treatment of the latter with excess thioacetic acid in DMF at 85° for 7 hours to yield after vacuum fractionation 24% of β-acetylmercaptomethyl-α-tetronic acid V: m.p. 70-74°, λ_{max}^{EtOH} 240 mμ, (ε=14,550), λ_{max}^{KBr} 3.03, 5.80, 5.92, 7.95, 8.70 μ. NMR: 3 protons at 7.60τ (acetyl), 2 at 5.30τ (ring CH_2), 2 at 6.16τ (exocyclic CH_2), 1 at 2.63τ (enol). Found: C, 44.81; H, 4.21; S, 17.34; CH_3CO, 22.66. Treatment of a tetramethylenesulfone solution of V with dry ammonia in the presence of air resulted in separation of the crystalline diammonium salt of the disulfide VI.[7] The latter was converted to the free dienol VI by acidification of its ice-cold aqueous solution, extraction with ethyl acetate and recrystallization of the extracted solid from t-butanol, overall yield from II 27.6%, m.p. 131-132°, λ_{max}^{EtOH} 244 mμ (ε=22,600), λ_{max}^{KBr} 3.10, 5.80, 5.90, 8.00, 8.77 and 12.90 μ. Mobility toward the anode in pH 7.0 buffer (collidine acetate, 0.05 M in acetate, 14 V/cm, 3 hours): 1.6 cm. Found: C, 41.17; H, 3.97; S, 22.02. Its dimethyl ether melted at 59°; NMR: 6 protons at 5.95τ (methyls), 4 at 6.36τ (bridge methylenes), 4 at 5.23τ (ring

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6 The quaternary salt IV when dissolved in water soon deposits crystals showing a violet $FeCl_3$ reaction, m.p. after sublimation (170°/0.001 mm) 242°, λ_{max}^{EtOH} 241 mμ (ε= 20,400), λ_{max}^{KBr} 3.04, 5.80 (d), 8.75, 10.06, 10.16 and 12.96 μ (spectrum very similar to that of β-methyl-α-tetronic acid). Found: C, 50.60; H, 3.73. This compound is identical with the by-product of the condensation of pyruvic acid with dimethylamine hydrochloride and formaldehyde, which had been characterized by Mannich and Bauroth as a "$C_{15}H_{14}O_{10}$ Acid."[2] We have now found, on the basis of analytical figures and of the NMR spectrum of its reaction product with diazomethane: m.p. 98.5-99.5°, NMR: 6 protons at 6.03τ (methyls), 4 at 5.33τ (ring methylenes), 2 at 6.60τ (bridge methylene); found: C, 54.77; H, 5.04; CH_3O, 26.00, that the acid has the composition $C_9H_8O_6$ (dimethyl ether $C_{11}H_{12}O_6$) and the structure of a β-methylene-bis-α-tetronic acid VIII.

7 The diammonium salt of VI was most efficiently prepared by performing the reaction of IV with thioacetic acid in tetramethylenesulfone solution, removing excess reagent in vacuo and other impurities by precipitation with methylenedichloride prior to treatment with ammonia.

methylenes); λ_{max}^{EtOH} 233 mμ (ϵ=19,300), $\lambda_{max}^{CHCl_3}$: 5.68, 5.97, 6.90, 7.37, 7.95, 8.03, 8.25, 8.90, 9.06, 9.65, 9.92, 11.66 μ. Found: C, 46.13; H, 5.75; S, 17.71.

The disulfide VI had antimicrobial activity against a variety of organisms.[8] Thus it inhibited the growth of S. aureus (P 209) at a concentration of 0.4 μg/ml, that of penicillin resistant S. aureus isolates at 1.6-1.8 μg/ml and that of trichophyton mentagrophytes at 0.7 μg/ml.[9]

The β-mercaptomethyl-α-tetronic acid (VII) could be obtained only in poor yield, due to the tendency of its monoanion for oxidation or self-condensation to yield I. Small quantities of VII could be isolated from the reaction of III with NaSH or from the reaction of IV with H_2S in DMF by paper chromatography[10] or by high vacuum distillation (105° bath temp. at 0.0005 mm). λ_{max}^{EtOH} 242 mμ (ϵ=8,300), λ_{max}^{neat} 2.92, 3.86, 5.67, 5.97, 8.87 μ, positive $FeCl_3$ and nitroprusside reactions.

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8 The authors wish to thank Dr. P. Actor and Mr. H. Basch for the antimicrobial assays.

9 The dimethyl ether of VI was active against S. aureus at 19 μg/ml. Compounds I, III and VIII showed no activity at 100 μg/ml.

10 The mobility of VII in a n-butanol:acetic acid:water, 4:1:4, system was about 1.11 times that of I and 1.05 times that of VI.