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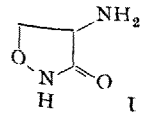
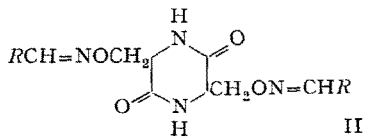
A Schiff Base of Cycloserine[1]

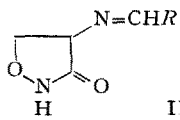
Cycloserine[2], a broad-spectrum antibiotic, has the structure D-4-amino-3-isoxazolidone (I). Its mode of action has been related[3] to its ability to react with pyridoxal, since it inhibits certain transaminase, decarboxylase and racemase enzyme systems which are known to be pyridoxal dependent[4]. In 1962, MICHALSKY[5] reported that DL-cycloserine reacts with pyridoxal and other aldehydes in boiling ethanol to form derivatives of cycloserine dimer (II) in nearly quantiative yields. On the basis of these results and some microbiological activity in the dimer[4b], he concluded that cycloserine derives its biological activity from its ability to form the dimer. He also concluded that a cycloserine Schiff base (III) is *not* the form in which pyridoxal is removed from enzyme systems since he was unable to isolate such a compound. Other workers[3] have suggested that a cycloserine Schiff base may be very labile and may rearrange rapidly into the dimer derivative (II).



I

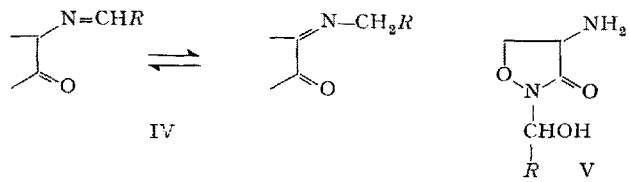


II



III

We were interested in testing the two above hypotheses; namely, that cycloserine does not readily form a Schiff base and that the Schiff base, if formed, rearranges rapidly to give a dimer derivative. We found that in *boiling* ethanol, D-cycloserine (I) reacts with 5-chlorosalicylaldehyde, which is known[6] to form Schiff bases with amino acids, to give a dimer derivative[7] (II, R = 2-hydroxy-5-chlorophenyl), m.p. 226–229°C, $[\alpha]_D^{22}$ +43.6° (c, 1.26 in dimethylformamide); ν, 3120 (N-H), 1670 (C=O), 1630 (C=N). This compound also showed the infrared bands at 1480 and 1330 cm^{-1} known[8] to be characteristic of diketopiperazines. When this reaction was carried out at room temperature, the Schiff base, N-(5-chlorosalicylidene)-D-cycloserine (III, R = 2-hydroxy-5-chlorophenyl), m.p. 144–146°C, $[\alpha]_D^{25}$ +130° (c, 1 in methanol); ν, 1710 (C=O), 1625 (C=N), was obtained in 84% yield. The Schiff base structure was shown by the following reactions. It was hydrolyzed rapidly at room temperature in 1.1 equivalents of 0.3N hydrochloric acid giving 5-chlorosalicylaldehyde in 76% yield and crystalline D-cycloserine in 48% yield. When an attempt was made to rearrange this product into the dimer derivative (II, R = 2-hydroxy-5-chlorophenyl) in boiling ethanol the crystalline reaction product, m.p. 155–156°C, was *not* the dimer derivative. Its lack of optical activity and its hydrolysis to 5-chlorosalicylaldehyde and DL-cycloserine showed it to be the Schiff base of DL-cycloserine. This is the first reported racemization of D-cycloserine and may be useful in the preparation of the racemate and consequently the L-isomer from the available D-isomer. Racemization of the asymmetric center at C-4 probably occurred *via* the tautomeric equilibrium IV. This has been postulated[9] as the mechanism by which pyridoxal-dependent racemases and transaminases act on amino acids. The racemization eliminates the possiblity that the adduct has the structure V (R = 2-hydroxy-5-chlorophenyl), which would result from attack by the aldehyde at the *ring* nitrogen, since it is difficult to see how V could be thermally racemized.



IV V

It *is* possible, however, that V is intermediate in the formation of dimer derivatives in boiling ethanol[5a]. Ethanolysis of the isoxazolidone ring of V gives an α-amino ester VI which might be expected to dimerize forming II. We prepared VI (R = CH_3, R' = 2-hydroxy-5-chlorophenyl) and found that on heating it with or without solvent, the dimer derivative was *not* formed. The oxime of 5-chlorosalicylaldehyde was obtained in 68% yield along with an insoluble gum. These products

[1] Cycloserine I.

[2] (a) F. A. KUEHL JR., F. J. WOLF, N. R. TRENNER, R. L. PECK, E. HOWE, B. D. HUNNEWELL, G. DOWNING, E. NEWSTEAD, K. FOLKERS, R. P. BUHS, I. PUTTER, R. ORMOND, J. E. LYONS, and L. CHAIET, J. Am. chem. Soc. *77*, 2344 (1955). – (b) P. H. HIDY, E. B. HODGE, V. V. YOUNG, R. L. HARNED, G. A. BREWER, W. F. PHILLIPS, W. F. RUNGE, H. W. STAVELY, A. POHLAND, H. BOAZ, and H. R. SULLIVAN, J. Am. chem. Soc. *77*, 2345 (1955).

[3] N. K. KOTSCHETKOW, Öst. Chem. Ztg. *62*, 276 (1961).

[4] (a) T. AOKI, Kekkaku *32*, 418 (1957); Chem. Abstr. *52*, 6622 (1958). – (b) T. AOKI, Kekkaku *32*, 544 (1957); Chem. Abstr. *52*, 7427 (1958), – (c) J. L. STROMINGER, Physiol. Rev. *40*, 87 (1960).

[5] (a) J. MICHALSKY, J. OPICHAL, and J. CTVRTNIK, Mh. Chem. *93*, 618 (1962). – (b) J. MICHALSKY, J. CTVRTNIK, Z. HORAKOVA, and V. BYDZOVSKY, Exper. *18*, 217 (1962).

[6] (a) J. C. SHEEHAN and V. J. GRENDA, J. Am. chem. Soc. *84*, 2417 (1962). – (b) F. C. MCINTIRE, J. Am. chem. Soc. *69*, 1377 (1947).

[7] All new compounds give acceptable elemental analyses. Melting points are uncorrected. Infrared bands are reported in cm^{-1}.

[8] H. BROCKMAN and H. MUSSO, Chem. Ber. *89*, 241 (1956).

[9] J. P. GREENSTEIN and M. WINITZ, *Chemistry of the Amino Acids* (John Wiley and Sons Inc., New York 1961), Vol. 1, p. 586.

can be explained by assuming β-elimination of the oxime from VI leaving an α-aminoacrylic ester which polymerized.

$$R'CH{=}NOCH_2\underset{\displaystyle NH_2}{\overset{|}{C}}HCOOR \qquad VI$$

We conclude from these results that D-cycloserine forms a Schiff base quite readily and that dimerization is *not* prerequisite to its reaction with aldehydes. Thus, Schiff base formation may well be the predominant means by which the antibiotic removes pyridoxal from enzyme systems. We have shown also that the dimer derivatives obtained in boiling ethanol are most likely due to dimerization of D-cycloserine *prior* to its reaction with the aldehyde. Pathways including Schiff base rearrangement or reaction at the isoxazolidone ring nitrogen atom are contraindicated. We are presently investigating the reaction between D-cycloserine and pyridoxal itself[10].

Zusammenfassung. Von D-Cycloserin ist die erste Schiffsche Base isoliert und charakterisiert worden. Bei Erhitzen in Äthylalkohol unterbleibt die Umlagerung in einen Cycloserindimerenabkömmling und ergibt Racemisierung. Es wird gefolgert, dass Aldehyde nicht auf das Stickstoffatom des Isoxazolidons reagieren und dass Dimerisation von Cycloserin *nicht* für die Reaktion mit Aldehyden erforderlich ist.

CH. H. STAMMER

Department of Chemistry, University of Georgia, Athens (USA), February 7, 1964.

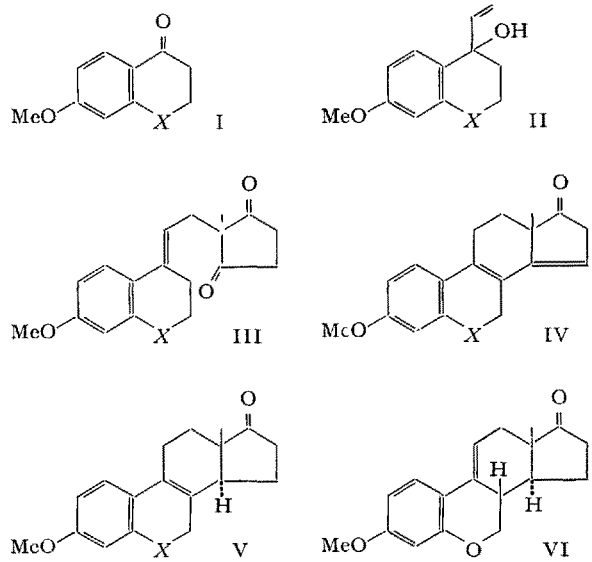
[10] *Acknowledgment.* We gratefully acknowledge the financial support of the National Institutes of Health, Grant number AI 05539-01. We thank Dr. W. F. RUNGE for a generous sample of D-cycloserine.

Total Synthesis of Derivatives of (±)-6-Oxa and Aza-Estrone

We wish to report extensions of our total syntheses of estrone, equilenin, and related compounds[1,2] to several corresponding 6-oxa- and -aza-analogs, and the conversion of (±)-17β-hydroxy-3-methoxy-6-oxa-estra-1,3,5-(10)-triene to compounds of the (±)-19-nor-6-oxa-testosterone series.

The ketones (I; $X=O$ and $C_6H_5SO_2N$)[3,4], made by cyclodehydration of the corresponding 3-substituted propionic acids, were used as starting materials. Their structures were confirmed by examining the aromatic proton signals in the proton nuclear magnetic resonance spectra of the first substance and the quinolone (I; $X=NH$) formed by acid hydrolysis of the second. The spectrum of the chromanone shows a doublet centered at 2.17 τ[5] (J 8.5 c/sec) assigned to the C_5-proton resonance split by coupling with the C_6-proton, a doublet at 3.59 τ (J 2.5 c/sec) assigned to the C_8-proton resonance split by coupling with the C_6-proton, and a pair of doublets centered at 3.43 τ assigned to the C_6-proton resonance split by coupling with both the C_5 and C_8-protons ($J_{6,5}$ and $J_{6,8}$ 9 and 2 c/sec, respectively). The spectrum of the quinolone (I; $X=NH$) is broadly similar with doublets centered at 2.18 and 3.84 τ (J 8.9 and 2.5 c/sec, respectively), and a pair of doublets at 3.67 τ ($J_{6,5}$ and $J_{6,8}$ 9 and 2.2 c/sec, respectively). These patterns are related to that found in the spectrum of the tetralone (I; $X=CH_2$) as two doublets centered at 2.01 and 3.31 τ (J 8.4 and 2.4 c/sec, respectively) and a pair of doublets at 3.18 τ (J 8.8 and 2.5 c/sec).

The chromanone (I; $X=O$) was converted by vinylmagnesium chloride in tetrahydrofuran, into the alcohol (II; $X=O$) (not obtained pure), and thence, by potassium hydrogen carbonate catalysed condensation with 2-methylcyclopentane-1,3-dione in methanol, into the seco-oxasteroid (III; $X=O$)[6], m.p. 107–111°, λ_{max} 264.5 and 309 mμ (ε 15 500 and 9400). Cyclodehydration of the last compound in ethanolic hydrochloric acid afforded the oxa-estrapentaene (IV; $X=O$)[7], m.p. 148–152°, λ_{max} 245 and 331 mμ (ε 16 700 and 21 700), which was selectively hydrogenated in benzene over 2% palladized calcium carbonate to the oxa-estratetraene (V; $X=O$), m.p. 146–151°, λ_{max} 286.5 and 307 mμ (ε 9000 and 10,500). Boiling ethanolic hydrochloric acid transformed the oxa-estratetraene into the $\Delta^{9(11)}$-isomer (VI), m.p. 175–179°, λ_{max}



[1] G. A. HUGHES and H. SMITH, Chem. and Ind. *1960*, 1022.
[2] G. H. DOUGLAS, J. M. H. GRAVES, D. HARTLEY, G. A. HUGHES, B. J. MCLOUGHLIN, and H. SMITH, J. chem. Soc. *1963*, 5072.
[3] W. H. PERKIN, J. N. RAY, and R. ROBINSON, J. chem. Soc. *1927*, 2094.
[4] J. T. BRAUNHOLTZ and F. G. MANN, J. chem. Soc. *1957*, 4166.
[5] Chemical shifts were measured downfield from tetramethylsilane as internal reference on a Varian A-60 spectrometer operating at 60 Mc/sec.
[6] Satisfactory analytical figures have been obtained for this and the other new compounds reported here.
[7] This and other racemic compounds described in the sequel are depicted by the enantiomorph having the 13-methyl group in the β-configuration.