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PARTIAL STRUCTURE OF THE ANTIBIOTIC ISOQUINOCYCLINE B

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A strain of Streptomyces aureofaciens was found by Martin et al.[1] to produce, under appropriate conditions, chlortetracycline and two other antibiotics, "β-Activity X" and "γ-Activity X". A similar finding was subsequently recorded and the names quinocycline B and isoquinocycline B,[2] respectively, applied; comparison of samples from both sources indicated the compounds to be identical.[3] The two isomers are reversibly interconverted in a manner reminiscent of the tetracycline-epitetracycline transformation.[4] We wish to report herein the results of preliminary structural studies of isoquinocycline B, the less active but more readily crystallized isomer.

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[1] J. H. Martin, A. J. Shay, L. M. Pruess, J. N. Porter, J. H. Mowat and N. Bohonos, Antibiotics Annual (1954-1955), p. 1020, Medical Encyclopedia, Inc., New York (1955).

[2] W. D. Celmer, K. Murai, K. V. Rao, F. W. Tanner, Jr. and W. S. Marsh, Antibiotics Annual (1957-1958), p. 484, Medical Encyclopedia, Inc., New York (1958).

[3] A. J. Shay, J. H. Martin and N. Bohonos, unpublished studies, utilizing paper chromatography as well as infra-red and ultraviolet spectroscopy. Isoquinocycline B was not available for direct comparison; however, equilibration of quinocycline B on paper strips produced a component with the same R_f as "γ-activity-X".

[4] A. P. Doerschuk, B. A. Bitler and J. R. D. McCormick, J. Amer. Chem. Soc. 77, 4687 (1955); C. R. Stephens, L. H. Conover, P. N. Gordon, F. C. Pennington, R. L. Wagner, K. J. Brunings and F. J. Pilgrim, Ibid. 78 1515 (1956).

The analytical data on isoquinocycline B hydrochloride,[5] recrystallized to constant analysis from ethanol and ethyl acetate and dried in vacuo over P_2O_5 at 80° for five hours, indicated $C_{36}H_{36}N_2O_{10}\cdot HCl.2H_2O$ [Calc.: C, 59.29; H, 5.67; N, 3.84; Cl, 4.86; m.w. 729.17. Found: C, 59.29; H, 5.89; N. 3.89; Cl, 4.88] although the range of analyses of a number of samples included $C_{33-38}H_{37-43}N_2O_{11-13}Cl$. Molecular weight determinations by the Menzies-Wright method varied from 347 to 412, indicating solvation; although there was usually no loss on drying at 140°, the Karl-Fischer moisture determination on several samples ranged from 2.01 to 5.80% (theory for 2 moles of H_2O, 4.93%). The ease of solvation of the antibiotic constantly plagued the structural studies; for example, if isoquinocycline B hydrochloride was recrystallized as above and then washed with acetone, it contained acetone which required treatment at 100° in vacuo for removal. Optically active, $[\alpha]_D^{25}$ + 12.0° (c 1, HOAc), isoquinocycline B had the following group analyses; Kuhn-Roth C-CH_3, 5.20% (theory for 2, 4.12%, for 3, 6.17%); N-CH_3, 2.32% (Calc. 1 N-CH_3, 2.06%); Van Slyke nitrogen, negative; O-CH_3, negative.

The spectral properties[5] of isoquinocycline B hydrochloride [$\lambda_{max}^{0.1N\ HCl}$ 231, 260, 292, 425-435 (broad) mu (log ϵ 4.74, 4.28, 4.03, 4.11, 4.12) $\lambda_{max}^{0.1\ N\ NaOH}$ 245, 280 (∽), 500 mu (log ϵ 4.72, 4.19, 4.18) λ_{max}^{KBr} 3.02, 3.25, 3.40, 3.70, 5.82s, 6.15, 6.23, 6.36, 6.80, 6.89, 7.05, 7.30, 7.63, 7.80, 7.91m, 8.02, 8.25, 8.40, 8.62, 8.90, 9.15, 9.53, 9.80s, 10.09, 10.26, 10.97, 11.25, 11.90, 12.20, 12.50, 12.75, 13.25, 13.62, 14.25, 14.47 μ] in direct comparison with those of a number of hydroxyanthraquinones indicated

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[5] The analytical data were obtained by Mr. L. Brancone and staff and the spectral data by Mr. W. Fulmor and staff; supplies of the antibiotic were furnished by the Fermentation Biochemistry Research Department, Lederle Laboratories. The authors wish to express their gratitude to these groups as well as to Dr. John S. Webb for helpful discussions.

a β,β'-dialkylated 1,5-dihydroxyanthraquinone (as shown by the uniform bathochromic shift of 6-7 mμ). This postulation was substantiated by the positive ferric chloride and alkaline hydrosulfite-oxygen tests for phenols and quinones, respectively, by the color reactions with piperidine,[6] titanium chloride,[7] and acetic anhydride-pyroboroacetate,[6] and by reductive acetylation with a refluxing zinc dust-sodium acetate-acetic anhydride mixture which yielded an impure amorphous solid, resistant to purification but exhibiting the ultra-violet absorption spectrum of a substituted anthracene, $\lambda_{max}^{CH_3OH}$ 340, 358, 376, 397 mμ ($E_{1cm}^{1\%}$ 43, 51, 57.5, 46.5).

The tetracyclic nature of the antibiotic was revealed via zinc dust distillation. Using a highly active form of zinc[8] the antibiotics and all of the chromophoric degradation products yielded naphthacenenoid distillates (by the visual spectra). In the case of isoquinocycline B a product was isolated, purified (alumina column chromatography using 60-70° petroleum ether-benzene, 1:4[9]) and shown to be different from naphthacene by comparisons of the infrared spectra and the X-ray powder diagrams,[10] although the visible spectrum, $\lambda_{max}^{benzene}$ 357, 377, 398.5, 420, 446,5, 476.5 mμ, was shifted bathochromically only 0.5 to 1.5 mμ from naphthacene. Since peri-substituted

[6] H. Brockmann, E. H. F. von Falkenhausen, R. Neeff, A. Dorlars and G. Budde, Ber. 84, 865 (1951).

[7] H. Brockmann and B. Franck, Ber. 88, 1792 (1955); F. Weygand and E. Csendes, Ibid. 85, 45 (1952).

[8] F. Kogl and W. B. Deijs, Ann. 515, 10 (1935); J. S. Webb, R. W. Broschard, D. B.Cosulich, W. J. Stein and C. F.Wolf, J. Amer. Chem. Soc. 79, 4563 (1957).

[9] J. A. Miller and C. A. Baumann, Cancer Research 3, 217 (1943).

[10] Our appreciation is extended to Mr. S. P. Kodama, American Cyanamid Co., Bound Brook, N. J., for these X-ray studies.

aromatics show a much larger spectral shift[11] and since the Kögl zinc left intact at least part of the peri-methyl group of the tetracyclines,[8] it was concluded that the present antibiotic lacked such substitution.

From the preceding data the antibiotic was considered to be a 1,6-dihydroxy-7,8,9,10-tetrahydro-5,12-naphthacenequinone with no more than one or two alkyl substitutents in β-positions. The premise that these substitutents were in the tetrahydro and not the aromatic end-ring was supported by the isolation of 3-methoxyphthalic anhydride, m.p. 144-150°C (sealed tube), (identity established by infrared spectra comparison with an authentic sample),[12] from the hot alkaline permanganate oxidation of a crude material obtained from the dimethyl sulfate methylation of the antibiotic.

Acid hydrolysis of isoquinocycline B hydrochloride with 1 N HCl at room temperature for twenty-four hours yielded a crystalline anhydro sugar ($C_8H_{14}O_4$) whose structure has been described elsewhere,[13] and a crystalline aglycone hydrochloride $[\alpha]_D^{25}$ +27.2° (c 1, HOAc), identified as isoquinocycline hydrochloride[2] by direct comparison.[3] Analytical data on material recrystallized from methanolic 1 N hydrochloric acid indicated $C_{25}H_{22}N_2O_6 \cdot HCl \cdot H_2O$ [Calc.: C, 59.93; H, 5.03; N, 5.59; Cl, 7.07; N-CH_3, 3.0; H_2O, 3.60. Found: C, 59.55; H, 4.74; N, 5.55; Cl, 6.93; N-CH_3, 1.19; H_2O, 4.20 (loss on drying)], although the range of empirical formulae from the analyses of a number of samples could include $C_{24-26}H_{23-25}N_2O_7Cl$; again, ease of solvation caused

[11] P. Korte, Angew. Chem. 63, 370 (1951).

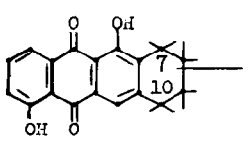
[12] Prepared by Mr. J. Petisi via the method of E. D. Amstutz, E. A. Fehnel and C. R. Neumoyer, J. Amer. Chem. Soc. 68, 349 (1946).

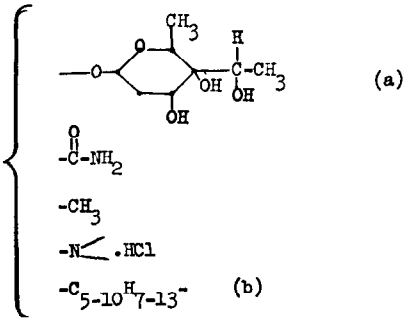
[13] J. S. Webb, R. W. Broschard, D. B. Cosulich, J. H. Mowat and J. E. Lancaster, J. Amer. Chem. Soc. 84 3182 (1962). Direct comparison of this compound and "Compound A" of Ref. 2, utilizing x-ray powder diagrams, infrared and n.m.r. spectra, mixture melting point, and optical rotations, showed them to be identical. The authors are indebted to Dr. W. D. Celmer of Chas. Pfizer & Co. for supplying a comparison sample of "Compound A".

difficulty in analysis and in attempts to determine the molecular weight. The spectral properties of the aglycone hydrochloride differed from those of the antibiotic only in the intensities of the ultraviolet absorption spectra maxima $[\lambda_{max}^{0.1\ N\ HCl}$ 230, 259, 291, 425, 435 mμ (log ε 4.71, 4.53, 3.99, 4.10, 4.11); $\lambda_{max}^{0,1\ N\ NaOH}$ 244, 280, 497 mμ (log ε 4.70, 4.18, 4.18)] and in the absence of an infrared maximum at 8.90 μ, all of which is consistent with the cleavage of a non-phenolic glycoside. No extensive search was made for one to three carbon fragments as possible products of this hydrolysis; however, no volatile carbonyl compounds were detected.

The two nitrogen atoms of isoquinocycline B also occur in the aglycone moiety (isoquinocycline). One was readily liberated as ammonia by acid or alkaline hydrolysis and was tentatively assigned to an amido group. The other was tertiary by the usual tests; lower alkyl substituents appeared to be absent (qualitative tests for N-methyl and N-ethyl were negative[14]) despite indication of N-Me in the group analyses. Classical degradations via the von Braun and Hofmann reactions lead to no interpretable results.

The following partial structure for isoquinocycline B hydrochloride was consistent with the limited data described above:





(a) See Reference 13 wherein the structure of the anhydro sugar was demonstrated and the nature of the parent glycose presumed.

(b) Uncertain because of analytical problems mentioned previously.

[14] F. Feigl and E. Silva, The Analyst. 82, 582 (1957).

At this point our investigations were temporarily interrupted. Since a possible relationship of the quinocyclines to the new tetracyclic class of compounds which includes pyrromycin, rhodomycin, cinerubin, and aklavin[15] was immediately apparent, we are reporting a summary of our quinocycline structure elucidation work, incomplete though it is.

15 (a) For a summary of these compounds see W. D. Ollis and I. O. Sutherland, Recent Developments in the Chemistry of Natural Phenolic Compounds, pp. 212-231, Pergamon Press, New York, 1961.

(b) The inclusion of the quinocyclines in this class has recently been suggested by M. W. Miller and F. A. Hochstein, J. Org. Chem. 27, 2525 (1962).