may be pulled slowly into strings, but it shatters on being struck. Higher molecular weight material is more brittle.

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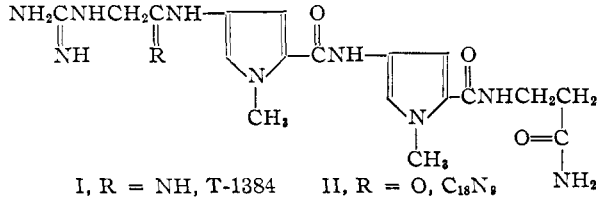
RECEIVED JANUARY 7, 1957

## THE STRUCTURE OF ANTIBIOTIC T-1384

*Sir:*

An antibiotic, designated T-1384 was isolated from an Actinomycetes type of organism by the Fermentation Biochemistry Department of these Laboratories.[1] This compound is identical with the antibiotic Netropsin,[2] $C_{32}H_{48}N_{18}O_4$.[3] Our data required the assignment of $C_{18}H_{26}N_{10}O_3$ for the empirical formula of T-1384. Subsequently, the latter empirical formula was reported for Netropsin,[4] sinanomycin,[5] and congocidine.[6] Reported herein are the data which indicate that T-1384 has structure I, β-[4-(4-guanidinoacetamidino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionamide.



I, R = NH, T-1384 II, R = O, $C_{18}N_9$

Mild alkaline hydrolysis of T-1384 gave the compounds $C_{15}H_{20}N_6O_3$, $C_3H_5N_3O$ and ammonia. The $C_3$ compound was identified as glycocyamidine by comparison with a known sample. Apparently the same $C_{15}H_{20}N_6O_3$ compound was obtained by hydrolysis of Netropsin,[3,4] and congocidine.[6] We have now established the structure of this compound as β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionamide.

Alkaline hydrolysis (0.5 *N* NaOH) of the above $C_{15}$ amide gave ammonia and the corresponding $C_{15}$ acid hemihydrate, m.p. 190–105° dec., $\lambda_{max}$. 0.1 *N* HCl: 285 mμ, ε = 20,300. *Anal.* C, 52.84; H, 5.70; N, 20.11; N-$CH_3$, 8.31; $H_2O$, 2.43; neut. eq., 379, 323. The $C_{15}$ acid and $C_{15}$ amide both gave a positive Bratton–Marshall test[7] for an aromatic amine while their N-acetyl derivatives gave negative tests. The ultraviolet absorption spectra of these N-acetyl derivatives and T-1384 ($\lambda_{max}$ 0.1 *N* HCl, 234 mμ, ε = 19,400, 300 mμ, ε = 22,400) were comparable indicating the presence of the same chromophoric system in these compounds.

Hydrolysis of the $C_{15}$ acid with 5 *N* sodium or barium hydroxide gave about *1.5 moles* of a $C_6$-$H_8N_2O_2$ compound, isolated as the $^1/_2$ $H_2SO_4$ salt, 187–202° dec. *Anal.* C, 37.79; H, 4.79; N, 14.54; S, 8.24; N-$CH_3$, 4.54; neut. eq., 99.8; $\lambda_{max}$. 0.1 *N* HCl: 260 mμ, ε = 9,800. The N-acetyl derivative of the $C_6$ compound (m.p. 200° dec. λmax. 0.1 *N* HCl: 232 mμ, ε = 13,200; 280 mμ, ε = 7,100) gave on heating 1 mole of carbon dioxide and an N-acetyl compound, $C_7H_{10}N_2O$, m.p. 119–120°. *Anal.* C, 60.36; H, 7.30; N, 19.82; N-$CH_3$, 7.85; N-acetyl, 31.32.

The presence of an N-methyl group, which had been indicated by our analysis of T-1384, was confirmed by the isolation and characterization of methylamine following the oxidation of the $C_6$ compound with acidic peroxide. The similarity of the ultraviolet absorption spectrum of the N-acetyl $C_5$ compound to N-ethylpyrrole,[8] the empirical formula, a positive Ehrlich test, and a positive Bratton–Marshall test[7] after hydrolysis suggested that the $C_5$ fragment was 3-amino-1-methylpyrrole.

The ease with which the $C_6$ compound was decarboxylated suggested an α-carboxyl group. Comparison of the $C_6$ fragment with a synthetic sample of 4 amino-1-methyl-2-pyrrolecarboxylic acid[9] showed them to be identical.

From the filtrates of the $C_6$ preparation was isolated β-alanine as its 2,4-dinitrophenyl derivative, m.p. 144–146°. *Anal.* C, 42.52; H, 3.69; N, 16.32. This derivative was identified by comparison with an authentic synthetic sample.

Hydrolysis data on the $C_{15}$ acid had shown it to contain two moles of 4-amino-1-methyl-2-pyrrolecarboxylic acid and one mole of β-alanine. Since the $C_{15}$ acid could not be decarboxylated readily and since it gave a positive test for an aromatic amine, the order of its fragments were postulated to be $C_6$—$C_6$—β-alanine. This order was also suggested by the ultraviolet absorption data. The structures of the $C_{15}$ acid and amide were established to be β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2 pyrrolecarboxamido]- propionic acid and the corresponding propionamide by comparison with synthetic samples.[9]

When T-1384 sulfate was treated with one equivalent of barium hydroxide at room temperature for 3 hours, there was produced ammonia and a new compound $C_{18}H_{25}N_9O_4 \cdot {}^1/_2H_2SO_4 \cdot {}^1/_2H_2O$, m.p. 200° dec. *Anal.* C, 43.88; H, 5.77; N, 25.12; S, 3.38. λ max. 0.1 *N* HCl: 234 mμ, ε = 19,600; 299 mμ, ε = 21,500. From spectral and hydrolytic data this $C_{18}N_9$ compound is postulated to be the N-guanidinoacetyl derivative of the $C_{15}$ amide, β-[4-(4-guanidinoacetamido-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionamide, structure II. A comparison of the $C_{18}N_9$ compound with a synthetic sample[9] of II confirmed its structure.

All features of the structure of T-1384 now have been established except the position and nature of the group giving rise to ammonia upon very mild hydrolysis. Potentiometric titration of T-1384

(1) S. De Voe, C. Ervin and N. Bohonos, unpublished data.

(2) Netropsin is the Trademark of Chas. Pfizer and Co. We wish to thank Dr. A. C. Finlay of Chas. Pfizer and Co. for a sample of Netropsin which was shown to be identical with T-1384 by chromatography and by spectral comparisons.

(3) A. C. Finlay, F. A. Hochstein, B. A. Sobin and F. X. Murphy, THIS JOURNAL, **73**, 341 (1951).

(4) E. E. van Tamelen, D. M. White, I. C. Kogon and A. D. G. Powell, *ibid.*, **78**, 2157 (1956).

(5) K. Watanabe, *J. of Antibiotics*, **9** (Ser. A), 102 (1956).

(6) M. Julia and N. Joseph, *Compt. rend.*, **243**, 961 (1956).

(7) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 527 (1939).

(8) R. A. Friedel and M. Orchin, "Ultraviolet Spectra of Aromatic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1951.

(9) M. J. Weiss, J. S. Webb and J. M. Smith, Jr., THIS JOURNAL, **79**, 1266 (1957).

salts showed only very strong basic groups. One very strongly basic function is the established guanidino group. The other is postulated to be an amidino group from its instability and strong basicity. The extreme instability of the amidine function suggests that the guanidinoacetamido group is the preferred location of this function. For these reasons structure I is postulated for the antibiotic T-1384.

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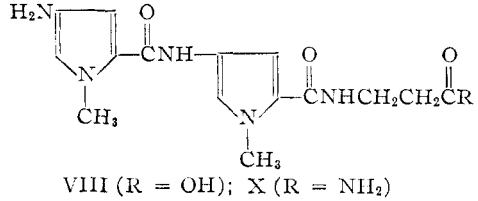
RECEIVED JANUARY 19, 1957

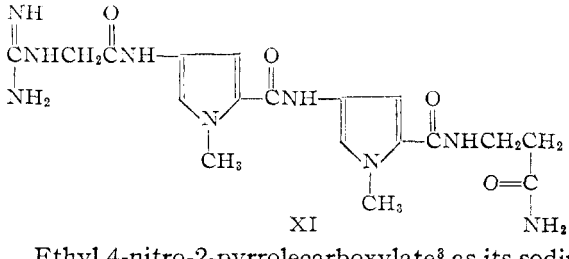
## THE STRUCTURE OF ANTIBIOTIC T-1384. SYNTHESIS OF THE DEGRADATION FRAGMENTS

*Sir:*

Waller and co-workers[1] have reported that the stepwise degradation of the compound designated in these Laboratories as antibiotic T-1384 and identical with Netropsin[2] gives four new compounds. They postulated that these are 4-amino-1-methyl-2-pyrrolecarboxylic acid (III); the tripeptide derived from two moles of III plus β-alanine, β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionic acid (VIII); the amide of VIII (X) and the N-guanidinoacetyl derivative of X (XI). We wish to report the synthesis of these four compounds, all of which were found to be identical with the corresponding T-1384 degradation fragments by comparison of their infrared absorption spectra and other appropriate methods.



VIII (R = OH); X (R = $NH_2$)



XI

Ethyl 4-nitro-2-pyrrolecarboxylate[3] as its sodium salt was N-methylated with methyl iodide in ethanol to give 89% ethyl 1-methyl-4-nitro-2-pyrrolecarboxylate (I), m.p. 113–114°. *Anal.* C, 48.2; H, 5.06; N, 14.0. Catalytic reduction of I gave 90% ethyl 4-amino-1-methyl-2-pyrrolecarboxylate (II) isolated as a $^1/_2H_2SO_4 \cdot {}^3/_4H_2O$ salt, m.p. 185° dec. *Anal.* C, 41.6; H, 5.77; N, 12.1; S, 7.00; $H_2O$, 5.64. Hydrolysis of II with aqueous barium hydroxide gave 61% 4-amino-1-methyl-2-pyrrolecarboxylic acid (III) isolated as the $^1/_2H_2SO_4 \cdot {}^1/_2H_2O$ salt, m.p. 202° dec. *Anal.* C, 36.1; H, 5.53; N, 14.1; S, 8.22.

The tripeptide VIII was synthesized by the following sequence. Alkaline hydrolysis of I gave 82% of the corresponding acid (IV), m.p. 195–197°. *Anal.* C, 42.0; H, 3.38; N, 16.6. By heating with excess thionyl chloride IV was converted to the acid chloride (V) which, without purification, was treated with β-alanine in sodium bicarbonate solution to yield 78% β-(1-methyl-4-nitro-2-pyrrolecarboxamido)-propionic acid (VI), m.p. 180–183°. *Anal.* C, 45.3; H, 4.85; N, 17.2. Catalytic reduction of VI as the sodium salt in aqueous solution gave the corresponding 4-amino compound which without isolation was condensed with the acid chloride V to give 57% (based on VI) β-[1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-propionic acid (VII), m.p. 250.5–251.5° dec. *Anal.* C, 49.4; H, 4.48; N, 19.7. Catalytic reduction of VII gave in good yield β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionic acid (VIII), isolated as the sesquihydrate, m.p. 235° dec. *Anal.* C, 50.3; H, 6.07; N, 19.9.

The third degradation product (X) was synthesized by treating the mixed carbonic anhydride[4] of VII in dimethylformamide solution with ammonia to give 70% β-[1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-propionamide (IX), m.p. 259–260° dec. *Anal.* C, 49.4; H, 4.61; N, 22.9. Hydrogenation of IX in dimethylformamide solution with palladium on carbon catalyst gave 82% β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionamide (X), m.p. 247–248° dec. *Anal.* C, 54.3; H, 6.09; N, 25.1.

Finally, synthesis of XI was accomplished by condensing X with guanidinoacetic acid originally by the mixed carbonic anhydride procedure,[4] which gave XI in 10% yield and later by the dicyclohexylcarbodiimide method[5] which afforded a 30% yield of the same product.[6] In both cases the material isolated was the $^1/_2H_2SO_4 \cdot H_2O$ salt of β-[4-(4-guanidinoacetamido-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]-propionamide (XI), m.p. 191–194° dec. *Anal.* C, 42.6; H, 5.41; N, 25.6; O, 22.4; S, 3.10.

We have just been informed that U. S. Patent 2,785,182 which covers compound XI above and was applied for on April 19, 1944, will be issued to C. W. Waller, M. J. Weiss and J. S. Webb on March 12, 1957.

(1) C. W. Waller, C. F. Wolf, W. J. Stein and B. L. Hutchings, THIS JOURNAL, **79**, 1265 (1957).

(2) Netropsin is the trademark of Chas. Pfizer and Co. for the antibiotic produced by *Streptomyces netropsis*. Structural studies on this antibiotic have been reported by Finlay and co-workers [*ibid.*, **73**, 341 (1951)] and by van Tamelen and co-workers [*ibid.*, **78**, 2157 (1956)]. Recently Watanabe [*J. Antibiotics* (A) **IX**, 102 (1956)] reported the antibiotic sinanomycin and Julia and Joseph [*Compt. rend.*, **243**, 961 (1956)] reported congocidine. Both these groups considered their antibiotics to be at least very similar to, if not identical with Netropsin.

(3) W. J. Hale and W. V. Hoyt, THIS JOURNAL, **37**, 2538 (1915).

(4) See J. R. Vaughan, Jr., *ibid.*, **73**, 3547 (1951).

(5) See J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(6) This latter preparation was carried out by Dr. A. S. Tomcufcik of these Laboratories.

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