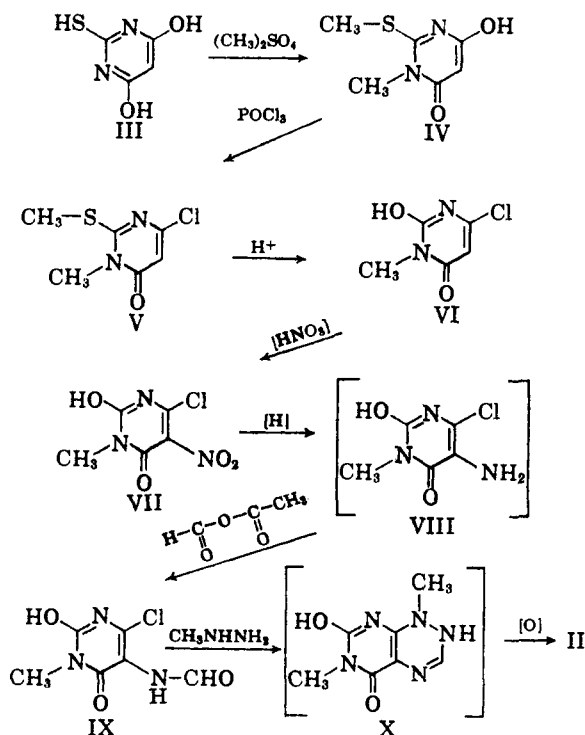We now wish to report the total synthesis of toxoflavin which confirms structure II in every respect. 2-Thiobarbituric acid[5] (III) was converted to 6-hydroxy-3-methyl-2-(methylthio)-4-(3H)-pyrimidinone (IV), m.p. 195–197° (calcd.: C, 41.8; H, 4.6; N, 16.3. Found: C, 42.1; H, 4.6; N, 16.0) via methylation. Chlorination of IV with phosphorus oxychloride in the presence of N,N-dimethylaniline

gave the corresponding chloro derivative (V) m.p. 111–112°, in 57% yield[6] (calcd.: C, 37.9; H, 3.7; N, 14.7. Found: C, 37.6; H, 3.8; N, 14.6). Compound V was hydrolyzed in dilute acid to yield 31–47% of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone (VI), m.p. 277–279° (calcd.: C, 37.5; H, 3.1; N, 17.4. Found: C, 37.3; H, 3.3; N, 17.4), which was nitrated readily below 25° to give 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone (VII), m.p. 195–197°, in 45% yield (calcd.: C, 29.3; H, 2.0; N, 20.5. Found: C, 28.8; H, 2.1; N, 20.2). Catalytic reduction of VII in methanol containing a small amount of aqueous ammonia, and then addition of formic–acetic anhydride to the concentrated reaction mixture gave 6-chloro-5-formamido-2-hydroxy-3-methyl-4(3H)-pyrimidinone (IX), m.p. 225–226°, in 43% yield (calcd.: C, 35.5; H, 3.0; N, 20.7. Found: C, 35.4; H, 3.2; H, 20.8). Compound IX then was refluxed with one equivalent of methylhydrazine in ethanol. The resulting precipitate was dissolved in a satu

(5) H. Michael, J. prakt. Chem., [2] 35, 456 (1887).
(6) All yields are calculated after purification.

rated ammonium sulfate solution, which was extracted with chloroform. Evaporation of the chloroform extract and recrystallization of the crude product from propanol-1 gave a 28% yield of toxoflavin, m.p. 172–173° dec., (lit.[3c] m.p. 171° dec.) as bright yellow plates (calcd.: C, 43.5; H, 3.7; N, 36.3. Found: C, 43.7; H, 3.7; N, 36.1).

The synthetic toxoflavin exhibited identical ultraviolet absorption [$\lambda_{max}^{pH\ 1.7}$ 257.5 mμ, (ϵ 16,400); 394 mμ, (ϵ 2,500)] and infrared absorption [λ_{max}^{KBr} (μ) 3.4 (w), 5.9 (s), 6.0 (s), 6.25 (s), 6.6 (s), 6.95 (m), 7.05 (m), 7.25 (w), 7.4(w), 7.8 (s), 8.1 (s), 8.35 (w), 8.8 (m), 9.6 (m), 10.4 (w), 10.9 (s), 11.55 (w), 12.35 (m), 13.0 (s), 13.8 (m) and 14.15 (m)] with that of the natural product.[3b,d,h]

Latuasan and Berends[3i] noted that toxoflavin apparently was identical with the antibiotic xanthothricin isolated by Machlowitz, et al.[7a] from a culture of a member of the genus Streptomyces. Comparison of an authentic sample of xanthothricin[7b] with synthetic toxoflavin spectrographically and in paper chromatographic systems confirmed this identity. (R_f values of both toxoflavin and xanthothricin in 95% ethanol and in 1-butanol–10% urea are 0.35 and 0.29, respectively.)

The present synthetic route should readily provide related compounds possessing interesting biological properties. An extension of this work currently is in progress. This study was made possible under the auspices of the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-3025.

(7) (a) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell and J. Charney, "Antibiotics and Chemotherapy," 4, 259 (1954); (b) this comparison was made possible by a generous gift of xanthothricin kindly provided by Dr. Frank J. Wolfe of Merck and Company, Inc., Rahway, New Jersey, to whom sincere thanks are due.

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A REVERSIBLE COMPLEX OF COBALTODIHISTIDINE WITH MOLECULAR NITRIC OXIDE

Sir:

Cobaltodihistidine (CoΦ_2) reversibly links nitric oxide in the molecular ratio 1:1 according to the reaction CoΦ_2 + NO $\rightleftarrows$ CoΦ_2NO, the stability constant of which, as determined by the measurements of the partial pressure of nitric oxide, has a value of approximately 10^4 at 20°. A solution 10^{-3} F of CoΦ_2 is practically colorless; it turns violet by adding nitric oxide and becomes colorless again by adding N_2. The violet solution exhibits maximum absorption at 455 mμ with a molar extinction coefficient of about 75. Solutions of CoΦ_2NO, acidified by perchloric acid, release nitric oxide quantitatively, and cobalt is found to be again in the bivalent state.

The addition and removal cycles of nitric oxide may be repeated many times in succession, but the quantity of nitric oxide added or removed diminishes after each cycle and simultaneously some oxidation to CoΦ_2^+ occurs. The oxidized form CoΦ_2^+ does not link nitric oxide. The be-

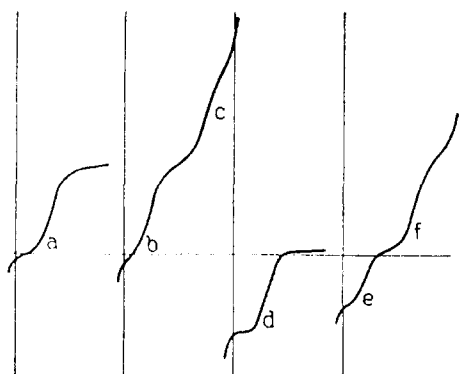Fig. 1.—(a) $Co\Phi_2^+$; (b), (c), $Co\Phi_2^+$ + NO; (d), $CoΦ_2$; (e), (f), $CoΦ_2$ + NO.

havior of $CoΦ_2^+$ and of $CoΦ_2$ in the presence of nitric oxide has been followed polarographically.

In phosphate buffer at pH 7.5, with histidine concentration 0.1 F and at 20°, solutions of $CoΦ_2^+$ (10^{-3} F) free of nitric oxide give (besides the reduction wave $CoΦ_2$ + $2e^-$ → Co^0 + $2Φ^-$ at $π_{1/2} \simeq -1.4$ v. $vs.$ S.C.E., which does not interest us here) a polarographic wave at $π_{1/2} = -0.21$ v. $vs.$ S.C.E. (Fig. 1, a) corresponding to the reduction $CoΦ_2^+$ + e^- → $CoΦ_2$. In the presence of nitric oxide, two waves are obtained on the polarogram (Fig. 1b, c), each having practically the same height as the one obtained in the absence of nitric oxide (Fig 1a), the half-wave potentials of which are, respectively, -0.16 and -0.42 v. $vs.$ S.C.E. (further indefinite waves, not of interest here, are caused by the second reduction of $CoΦ_2NO$ by the reduction of nitric oxide and of $CoΦ_2$ → Co^0).

Under the above-mentioned temperature, pH and [Φ] conditions, solutions of $CoΦ_2$ show on the polarograph an oxidation wave with $π_{1/2} = -0.20$ v. $vs.$ S.C.E. (Fig. 1d). In the presence of nitric oxide, this oxidation wave shifts its half-wave potential to -0.16 v. (Fig. 1e) and a new reduction wave appears with $π_{1/2} = -0.40$ v. $vs.$ S.C.E. (Fig. 1f), its height being practically equal to that of the oxidation wave of $CoΦ_2$ (Fig. 1d) in the absence of nitric oxide. The oxidation wave ($π_{1/2} = -0.16$ v.) (Fig. 1e) is always smaller, though not quantitatively reproducible, because of uncontrolled oxidation ($CoΦ_2NO$ → $CoΦ_2^+$ + NO^-) catalyzed by the electrode surface. These results may be explained as follows: the $CoΦ_2$ oxidation wave and the $CoΦ_2^+$ reduction wave in the presence of nitric oxide (Fig. 1e, b), both with $π_{1/2} \simeq -0.16$ v., are relative to the reaction $CoΦ_2^+$ + NO + e^- ⇌ [$CoΦ_2NO$]. The reduction waves (Fig. 1f, c), obtained from solutions of $CoΦ_2$ and $CoΦ_2^+$ in the presence of nitric oxide, are attributed to the reduction [$CoΦ_2$-NO] + e^- → [$CoΦ_2NO$]$^-$. In the case of the reduction of $CoΦ_2^+$ in the presence of nitric oxide, this wave (Fig. 1c) is caused by the reduction of $CoΦ_2$ NO formed at the electrode surface as a reaction product of nitric oxide contained in the solution, with $CoΦ_2$ obtained in the first reduction process $CoΦ_2^+$ + e^- → $CoΦ_2$. In fact, measurements of the partial pressure of nitic oxide on a solution of $CoΦ_2^+$, and spectrophotometric measurements on a solution of $CoΦ_2^+$ in the presence and absence of nitric

oxide, exclude interactions between $CoΦ_2^+$ and nitric oxide.

The reduction of $CoΦ_2NO$ (Fig. 1c, f) leads to a complex [$CoΦ_2NO$]$^-$ (still including nitric oxide inside). This is confirmed by the coulometry of a solution of $CoΦ_2NO$ made at -0.50 v. $vs.$ S.C.E., which eliminated proportionally both waves, the oxidation one (Fig. 1e) and the reduction one (Fig. 1f). With the coulometry carried out at -0.75 v. $vs.$ S.C.E., the polarogram finally shows only the $CoΦ_2$ oxidation wave (Fig. 1d). This means that at the latter potential, a further reduction of nitric oxide within the complex is obtained and $CoΦ_2$ is produced again.

The results show that $CoΦ_2$ exhibits with nitric oxide the same "carrier" property that it shows with oxygen.[1]

(1) J. Z. Hearon, D. Burk and A. L. Schade, *J. Natl. Cancer Inst.*, 9, 337 (1949).

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DEGRADATION OF THIOSTREPTON. DERIVATIVES OF 8-HYDROXYQUINOLINE

Sir:

Earlier communications from these laboratories have described the isolation and general properties of the antibiotic thiostrepton[1,2,3] and more recently, the isolation of three thiazole amino acid derivatives.[4,5] The antibiotic also contains an additional chromophoric system, and we now present evidence from parallel pyrolytic and hydrolytic degradations concerning this part of the molecule.

Pyrolysis of thiostrepton at 250–350° and 0.2 mm. pressure yielded, in addition to the diketopiperazine of alanine and isoleucine, two crystalline phenolic bases characterized by deep green ferric chloride reactions. The first, m.p. 90–95°, $λ_{max}^{EtOH}$ 242 mμ (42,500) and 314–316 mμ (3,500) was not obtained completely pure, but its molecular formula $C_{11}H_{11}ON$ was assured by high resolution mass spectrometry (Found: mol. wt., 173.142; calcd. for $C_{11}H_{11}ON$: mol. wt., 173.139).[6] Its identification as a derivative of 8-hydroxyquinoline was suggested by the characteristic ultraviolet spectrum, solubility in hexane, ferric chloride test, formation of an orange chloroform-soluble copper complex, and a close similarity in infrared spectrum with that of 4-methyl-8-hydroxyquinoline. It was identified as 4-ethyl-8-hydroxyquinoline (I) by comparison with an authentic sample, m.p. 92–93°; *Anal.*

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(2) J. F. Pagano, M. J. Weinstein, H. A. Stout and R. Donovick, *ibid.*, 1955–1956, p. 554.

(3) B. A. Steinberg, W. P. Jambor and Lyda O. Suydam, *ibid.*, 1955–1956, p. 562.

(4) G. W. Kenner, R. C. Sheppard and C. E. Stehr, *Tetrahedron Letters*, 23 (1960).

(5) M. Bodanszky, J. T. Sheehan, J. Fried, N. J. Williams and C. A. Birkhimer, *J. Am. Chem. Soc.*, 82, 4747 (1960).

(6) We are grateful to Mr. J. H. Beynon and Mr. A. E. Williams of Dyestuffs Division, Imperial Chemical Industries, Ltd., Blackley, Manchester, England, for this determination.