clines produced. We therefore feel that the dimethylaminonaphthacenes, **4** and **5**, are not true intermediates in the biosynthesis of the tetracyclines but may be accessible to the biosynthetic pathway by loss of the dimethylamino group.[9]

It would appear from the high yield achieved in the conversion of **3** to 7-chlorotetracycline, in spite of the number and complexity of the steps involved, that **3** is a normal intermediate[10] in the biosynthesis of the 6-methylated tetracyclines. By analogy we assume that **2** plays a similar role in the biosynthesis of the 6-nonmethylated tetracyclines.

(9) While incorporation of C^{14} methyl groups into the tetracyclines could be accounted for by exchange of methyls with methionine, we have not observed such exchange in a closely related system; namely, in the biological rehydration of 5a,6-anhydro-7-chlorotetracycline by *S. aureofaciens* V828 in the presence of C^{14}-methyl-labeled methionine we obtained 7-chlorotetracycline containing no C^{14}.

(10) As the usual simplification, we consider the isolated compounds to be the equivalent of their conjugated or enzyme-complexed forms which would presumably be involved in the biosynthetic processes as true intermediates.

LEDERLE LABORATORIES
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK
J. R. D. MCCORMICK
SYLVIA JOHNSON
NEWELL O. SJOLANDER

RECEIVED MARCH 18, 1963

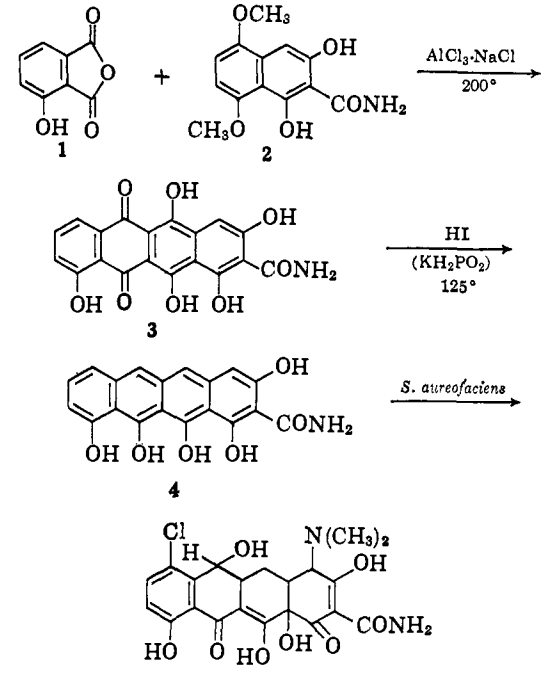
BIOSYNTHESIS OF THE TETRACYCLINES. VI.[1] TOTAL SYNTHESIS OF A NAPHTHACENIC PRECURSOR: 1,3,10,11,12-PENTAHYDROXYNAPHTHACENE-2-CARBOXAMIDE

Sir:

We have reported previously[1] the biological conversion by *Streptomyces aureofaciens* of 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide (**4**) and several related compounds to tetracycline antibiotics. In that work the naphthacene derivatives were prepared from tetracycline antibiotics by known degradation methods. An attempted synthesis of one of these compounds, 6-methyl-1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide, has been reported,[2a,b] but to date no total synthesis of an intermediate biologically convertible to a tetracycline has been reported.[3] We now wish to report the total synthesis of **4** and the biological conversion of the synthetic substance to 7-chloro-6-demethyltetracycline.

The synthesis of **4** was accomplished by condensing 3-hydroxyphthalic anhydride[4] (**1**) with 1,3-dihydroxy-5,8-dimethoxynaphthalene-2-carboxamide[5] (**2**) and reducing the resulting naphthacenequinone (**3**).

The condensation of **1** with **2** was accomplished by fusion of an intimate mixture of 2 mmoles of each with 10 g. of anhydrous aluminum chloride and 2 g. of sodium chloride at 200° for 2 hr. The melt was cooled, decomposed with 3 *N* hydrochloric acid and the resulting black crude solid was washed thoroughly with water. The desired product, 1,3,5,10,12-pentahydroxynaphthacene-6,11-quinone-2-carboxamide (**3**) was separated by dissolving the crude solid in 1:1 dimethylformamide–water and extracting into 1:10 triethylamine–chloroform. The quinone (146 mg., 19%) was isolated by evaporating the deep blue extract. It was recrystallized from dimethylformamide–triethylamine. *Anal.* Found for $C_{19}H_{11}NO_8$: C, 59.4; H, 3.06; N, 3.54; m.p. 355–360° dec.; λ_{max} in mμ (ϵ): 303 (23,400), 384 (4,220), 404 (5,070), 578 (13,900), 627 (25,900).[6]



7-Chloro-6-demethyltetracycline

The quinone was reduced by dissolving the total product from the preceding step in 5 ml. of phenol, adding 2 ml. of 58% hydriodic acid and 200 mg. of potassium hypophosphite, and refluxing for 3 hr. The product crystallized on cooling to form 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide.[7] *Anal.* Found for $C_{19}H_{13}NO_6$: C, 65.2; H, 3.92; N, 3.92; m.p. 290–320° dec., λ_{max} in mμ (ϵ): 231 (23,200), 264 (28,900), 282 (31,100), 337 (15,800), 394 (16,100), 490 (16,400).[6] The infrared spectrum (KBr disk) was identical with that of authentic **4**.

Authentic **4** was prepared from 6-demethyltetracycline following the procedure used by Green, Wilkinson and Boothe[8] on tetracycline. 6-Demethyltetracycline was reduced to 4-dedimethylamino-6-demethyl-12a-deoxytetracycline with zinc and acetic acid. The isolated crude product [*Anal.* Found for $C_{19}H_{17}NO_7$: C, 61.1; H, 5.00; N, 3.30; λ_{max} (0.1 *N* hydrochloric acid in methanol) in mμ (ϵ): 261 (18,600), 353 (12,000), 431 (3,030), 452 (shoulder) (2,350)] was brominated in tetrahydrofuran with N-bromosuccinimide and the resulting crude 12a-bromo derivative was dehydrobrominated in pyridine at 100° to yield 4a,12a-anhydro-4-dedimethylamino-6-demethyltetracycline. *Anal.* Found for $C_{19}H_{15}NO_7$: C, 61.2; H, 4.26; N, 3.65; λ_{max} (0.1 *N* hydrochloric acid in methanol) in mμ (ϵ): 273 (10,300), 402 (34,900), 420 (32,900). This product was dissolved in phenol and dehydrated with hydrochloric acid in acetic acid to yield crystalline **4**. *Anal.* Found for $C_{19}H_{13}NO_6$: C, 64.6; H, 3.91; N, 3.89; λ_{max} in mμ (ϵ): 232 (22,800), 264 (28,600), 281 (31,000), 333 (14,000), 394 (15,300), 490 (16,700).[6]

(1) Paper V: J. R. D. McCormick, S. Johnson and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1692 (1963).

(2) (a) Y. T. Huang, H. C. Tsung, L. H. Tai, H. Y. Sheng and T. Y. Tu, *Hua Hsüeh Hsüeh Pao*, **24**, 311 (1958); (b) Y. T. Huang, *Tetrahedron*, **11**, 52 (1960).

(3) J. H. Boothe and co-workers [*J. Am. Chem. Soc.*, **81**, 1006 (1959)] and H. Muxfeldt [*Chem. Ber.*, **92**, 3122 (1959)] have reported the total syntheses of 5a,6-anhydro-7-chloro-4-dedimethylamino-6-demethyl-12a-deoxytetracycline and 5a,6-anhydro-4-dedimethylamino-12a-deoxytetracycline, respectively. We have found that these compounds are not biologically converted to tetracycline antibiotics in our system (unpublished work).

(4) E. L. Eliel, A. W. Burgstahler, D. E. Rivard and L. Haefele, *J. Am. Chem. Soc.*, **77**, 5092 (1955).

(5) Z. Budesinsky and A. Svab, *Chem. Listy*, **51**, 1333 (1957).

(6) Absorption spectra were determined in 98% sulfuric acid containing 0.1% of boric anhydride. Solutions were allowed to stand 30 min. before spectra were determined.

(7) The condensation of 3-hydroxyphthalic anhydride with the naphthaleneamide would be expected to generate two isomeric products, a 7-hydroxynaphthacene and a 10-hydroxynaphthacene. Separation of these two isomers presumably occurred either in the triethylamine–chloroform extraction or in the crystallization to the final product.

(8) A. Green, R. G. Wilkinson and J. H. Boothe, *J. Am. Chem. Soc.*, **82**, 3946 (1960).

The biological conversion of the synthetic precursor, **4**, to 7-chloro-6-demethyltetracycline was carried out using *S. aureofaciens* V828 by the method previously described.[1] The product was identified by paper chromatography and represented a 7.9% yield (microbiological assay) based on precursor added to the fermentation. A control conversion of authentic **4** carried out simultaneously resuted in a yield of 8.4% of 7-chloro-6-demethyltetracycline.

The total synthesis of the naphthacenic precursor, **4**, and the biological conversion to 7-chloro-6-demethyltetracycline constitute a potential chemical-biological route to new tetracycline antibiotics involving, in the chemical steps, only aromatic chemistry.[9] Investigation of this route as a source of semisynthetic new tetracycline antibiotics is in progress.

(9) L. H. Conover and co-workers [*J. Am. Chem. Soc.*, **84**, 3222 (1962)] recently have reported the total synthesis of (±) 6-demethyl-6-deoxytetracycline by an essentially aliphatic approach.

LEDERLE LABORATORIES
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

J. R. D. MCCORMICK
JULES REICHENTHAL
SYLVIA JOHNSON
NEWELL O. SJOLANDER

RECEIVED MARCH 18, 1963

INFRARED ABSORPTION BY PEROXY-NITROGEN TRIOXIDE FREE RADICAL IN THE GAS PHASE

Sir:

We have observed a new absorption band with a maximum occurring at 1840 cm.$^{-1}$, which we assign to the peroxy-free radical, OONO. This observation was made by means of a prism-grating spectrophotometer (Beckman IR-7) in conjunction with a pair of long path (80 m.) multireflection cells. Pressure of nitric oxide in the 270-l. sample cell was between 0.5 and 2.5 mm. and pressure of oxygen varied from 10 to 50 mm. Oxygen reacts slowly and irreversibly with nitric oxide at these pressures and at room temperature to produce nitrogen dioxide.

$$2NO + O_2 \longrightarrow 2NO_2 \qquad (1)$$

Nitrogen dioxide and nitric oxide react rapidly and reversibly to produce dinitrogen trioxide.

$$NO + NO_2 \rightleftharpoons N_2O_3 \quad K_{25^\circ} = 0.47 \text{ atm.}^{-1}, \quad \Delta H^\circ = -10.3 \text{ kcal./mole} \qquad (2)$$

The peroxy-free radical presumably is formed by the rapid reversible reaction

$$O_2 + NO \rightleftharpoons OONO \qquad (3)$$

The absorption spectra of NO, N_2O_3, and OONO are given in Fig. 1. All three absorb infrared radiation in the same spectral region, but they can be distinguished on the basis of their behavior as a function of time and by differences in optical density where their absorption maxima do not overlap. When nitric oxide alone was placed in the absorption cell, and oxygen quickly added, the P branch of nitric oxide was instantly enhanced. Within a few minutes enough dinitrogen trioxide was formed by reactions 1 and 2 to cause a further and a different enhancement in this spectral region. By correcting for absorption due to NO and N_2O_3, the difference spectrum shown in Fig. 1 was obtained. (This procedure is complicated to some extent by the noticeable adsorption of nitric oxide on the surface of the optical cell. This adsorbed material could be displaced either by added oxygen or nitrogen. The amount of nitric oxide actually present was given by the non-overlapped R branch.)

A series of runs was made with constant initial nitric oxide and varied pressures of added oxygen. The new absorption is linear in oxygen, as can be seen from Fig. 2. Similarly, a series of runs was made with varied initial nitric oxide and constant oxygen. The optical density of the new band is linear in nitric oxide, Fig. 3.

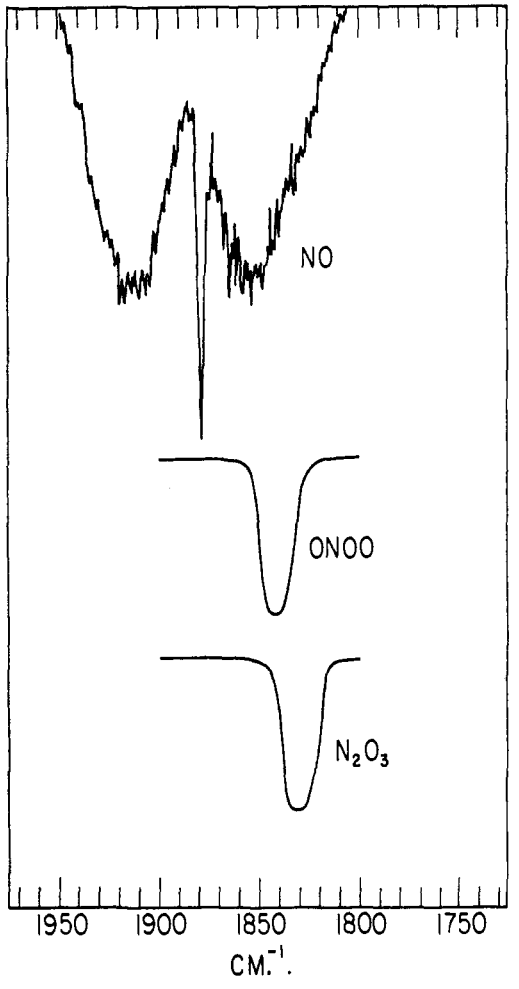


Fig. 1.—Absorption spectra as observed in this study.

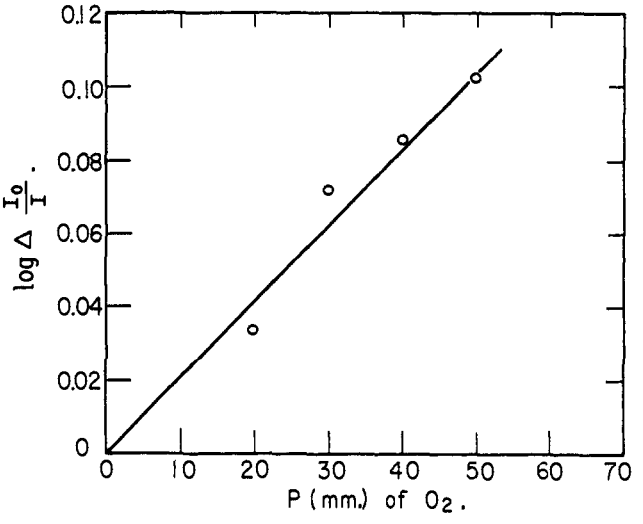


Fig. 2.—First-order dependence of optical density at 1840 cm.$^{-1}$ on oxygen for constant nitric oxide. Corrections have been made for absorption by NO and by N_2O_3.

The evidence cited in support of identifying the compound as NO_3 is the first-order dependence on both NO and O_2, and the time behavior of the new band. The structural assignment OONO, rather than that of the symmetrical nitrate radical O—N<O, O>, is