ion exchange chromatography.[7] Glucose-1-$d_1$ and -1-$t_1$ were phosphorylated with hexokinase.[4] The isotopic purity of the deuterated NADP and glucose-1-$d_1$ were confirmed by nmr to be better than 90%.[4] Doubly sonicated particles ($P_3$) and crude reductase ($S_2$) were prepared from adrenal mitochondria, reconstituted in the same proportion as found in the crude sonicate and incubated with DOC.[1] The incubation with NADP(D)D gave 5.25 nmol of CORT/ml/10 min ± 0.29 (SEM) while NADPH gave 5.75 ± 0.30. On the basis of the above consideration,[4] this failure to observe an isotope effect suggests that the reaction does not proceed through Scheme II.

It could well be that I failed to find any isotope effect on the rate because of the relative impurity of the system. But the only difference which might affect the crude system as opposed to the more purified is that the crude may catalyze the exchange of the 4-D with the water, as has been observed by Popjak, *et al.*,[8] with liver-soluble fractions. In such a case the actual reacting species might not be deuterated at all. To determine the significance of exchange, NADP(T)T ($3.39 \times 10^5$ dpm per incubation) was added to the mixture and incubated as above except in a nitrogen atmosphere rather than air. Only 1710 dpm ± 300 were found in the water in the presence of enzyme and 1360 dpm ± 400 in its absence. This would indicate that the enzyme caused an exchange of 350 dpm or 0.1%. Clearly exchange is not important in this preparation and the reacting species is actually NADP(D)D.

These results therefore indicate that the rate-limiting step is not the rupture of the C–H bond in the dihydronicotinamide ring nor the direct transfer of a hydrogen from NADPH. A second electron chain, other than the one purified by Omura, *et al.*, may be involved, but except for the work of Sih, *et al.*, there is little evidence to indicate this latter possibility.

(7) A. Kornberg and B. L. Horecker, *Biochem. Prep.*, **3**, 24 (1953).
(8) G. Popjak, D. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, **236**, 1934 (1961).

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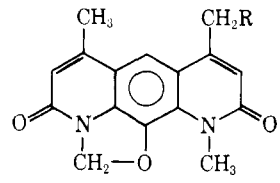
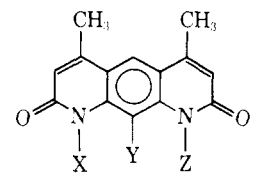
## Nybomycin. III. A Revised Structure[1,2]

*Sir:*

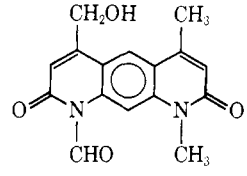
The antibiotic nybomycin was isolated some years ago in two laboratories from streptomyces cultures;[3,4] it is quite active against Gram-positive bacteria, but is insoluble. Deoxynybomycin, originally described by us as a degradation product of nybomycin,[5] was very recently reported by Umezawa, *et al.*, as an antibiotic produced by *Streptomyces hyalinum* n. sp. Hamada *et* Yakayama;[6] those authors' data indicated somewhat higher activity for deoxynybomycin than for nybomycin itself. We wish to assign here revised structures to nybomycin (**1**) and deoxynybomycin (**2**) and to report a partial synthesis of the latter compound.



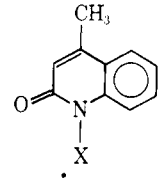
**1**, R = OH
**2**, R = H
**10**, R = -OCO(CH$_2$)$_2$CH$_3$
**12**, R = D
**13**, R = -OCOCF$_3$



**3**, X = Y = Z = H
**11**, X = H; Y = OH; Z = CH$_3$



**4**



**5**, X = -CHO
**6**, X = H
**7**, X = -CH=CHCOOC$_2$H$_5$
**8**, X = -CH=CHCOOH
**9**, X = -CH=CH$_2$

The 1,8-diazaanthracene ring system assigned to nybomycin[5] was confirmed earlier[1] by the total synthesis (from *m*-xylene) of **3**, obtained *via* phosphorus–hydriodic acid reduction of deoxynybomycin (**2**).[5]

The structure (**4**) we assigned earlier to nybomycin became untenable with our synthesis of the previously unknown *N*-formyl-2-lepidone (**5**). 2-Lepidone (**6**), prepared by the method of Camps,[7] was treated with ethyl propiolate and sodium methoxide in toluene at reflux to give ethyl 2-lepidone-1-acrylate (**7**, $C_{15}H_{15}NO_3$, mp 116–117°)[8] in 56% yield. The ester (**7**) was saponified to the acid (**8**, $C_{13}H_{11}NO_3$, mp 227–230°, 75% yield),[8] which was decarboxylated at 190° over copper chromite in quinoline to give *N*-vinyl-2-lepidone (**9**, $C_{12}H_{11}NO$, mp 69–70°, 77% yield).[8,9] Ozonolysis of **9** in methanol gave **5** ($C_{11}H_9NO_2$, mp 143–145°)[8,9] in 56% yield. The properties of **5** differ from those of nybomycin and deoxynybomycin in a number of respects: the *N*-formyl group of **5** is acid sensitive, deoxynybomycin is not; the *N*-formyl infrared absorption of **5** occurs at 1725 cm$^{-1}$, no absorption above 1665 cm$^{-1}$ is found for **1** and **2**; finally, the nmr signal ($CDCl_3$) for the *N*-formyl proton of **5** is found at $\delta$ 9.93, while the lowest field absorption (in $CDCl_3$) of nybomycin *n*-butyrate (**10**, $C_{20}H_{22}N_{22}O_5$, mp 203–204°)[8,9] is at $\delta$ 7.35.

The two-proton signal at $\delta$ 6.36 in the spectrum of **10** suggested a methylene group of the X–CH$_2$–Y type. Oxidation of **2** with manganese dioxide in formic acid at 3° converted **2** to **11** [$C_{15}H_{14}N_2O_3$, mp 334–335° dec][8,9] in 28% yield. The latter compound (**11**) was

(1) Paper II: G. Leadbetter and K. L. Rinehart, Jr., *Can. J. Chem.*, **43**, 1625 (1965).
(2) Portions of this work were presented at the 5th International Symposium on the Chemistry of Natural Products, IUPAC, London, July 1968; Abstracts, p 79.
(3) F. Strelitz, H. Flon, and I. N. Asheshov, *Proc. Nat. Acad. Sci. U. S.*, **41**, 620 (1955).
(4) T. E. Eble, G. A. Boyack, C. M. Large, and W. H. DeVries, *Antibiot. Chemother.*, **8**, 627 (1958).
(5) K. L. Rinehart, Jr., and H. B. Renfroe, *J. Amer. Chem. Soc.*, **83**, 3729 (1961).
(6) H. Naganawa, T. Wakashiro, A. Yagi, S. Kondo, T. Takita, M. Hamada, K. Maeda, and H. Umezawa, *J. Antibiot.*, **23**, 365 (1970).
(7) R. Camps, *Arch. Pharm.*, **237**, 659 (1899).
(8) Microanalyses agree with the molecular formula shown.
(9) Low-resolution mass spectral data agree with the molecular formula shown.

then reconverted to **2** in 51% yield by treatment with methylene bromide and potassium carbonate in dimethylformamide at 100°, in a reaction constituting a partial synthesis of deoxynybomycin.

The question of which aromatic methyl of **2** bears a hydroxyl substituent in nybomycin was resolved conclusively by spin decoupling carried out on nybomycin in trifluoroacetic acid. Irradiation of the aromatic methylene signal at δ 5.58 sharpened the ring-proton absorption at δ 7.69, while irradiation of the aromatic methyl signal at δ 2.87 sharpened the ring-proton absorption at δ 7.16. The position of the latter signal is at the precise position expected for the pyridone ring proton of a 4-methyl-2-quinolone bridged 1,8 by a methylene bridge.[10-12] Hence, the aromatic methyl is on the same ring as the methylene bridge, as shown in **1**. In the accompanying report[10] we describe the total synthesis of deoxynybomycin (**2**).

**Acknowledgment.** This investigation was supported in part by Public Health Service Research Grants No. AI 01278 and AI 04769 from the National Institute of Allergy and Infectious Diseases. We also thank the Upjohn Co. for a sample of nybomycin.

(10) See accompanying manuscript: R. M. Forbis and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **92**, 6995 (1970).

(11) Both the pyridone ring proton and the methyl group protons in 2-lepidones are remarkably constant in their chemical shifts so long as the character of the N-substituent is unchanged (see Table I, accompanying report).[10] That it is in fact the 6-methyl which is found at δ 2.90 in the spectrum of **2** was verified by reduction of **1** with 47% deuterium iodide at reflux; the nmr spectrum of the product (**12**) shows a two-proton singlet at δ 2.98 (Ar-$CH_2D$) and a three-proton signal at δ 2.90.

(12) Upon standing in trifluoroacetic acid, nybomycin forms a trifluoroacetate ester (**13**), whose nmr spectrum retains the singlet peaks at δ 2.87 (3 H), 6.82 (2 H), and 7.16 (1 H), for the protons on the oxazolinopyridone rings, but shows shifted singlets at δ 4.45 (3 H), 6.03 (2 H), and 7.46 (1 H), for the protons on the trifluoroacetate-substituted ring.

(13) National Science Foundation Cooperative Graduate Fellow.

(14) National Science Foundation Predoctoral Fellow.

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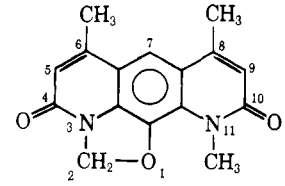
## Nybomycin. IV. Total Synthesis of Deoxynybomycin[1]

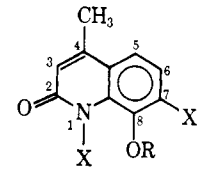
*Sir:*

Umezawa, *et al.*,[2] recently reported the isolation of the antibiotic deoxynybomycin from *Streptomyces hyalinum* n. sp. Hamada *et* Yakayama. That compound, which we assigned structure **1**,[1,3] had earlier been reported as a degradation product of the antibiotic nybomycin.[4] We report here the unambiguous synthesis of 6,8,11-trimethyl-4,10-dioxo-2*H*,4*H*,10*H*,-11*H*-pyrido[3,2-*g*]oxazolo[5,4,3-*ij*]quinoline (**1**) and its complete identity with deoxynybomycin.

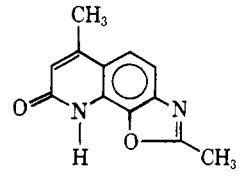
Our synthetic route began with the acylation of commercially available *o*-anisidine with diketene[5] to



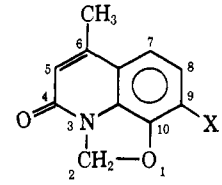
**1**



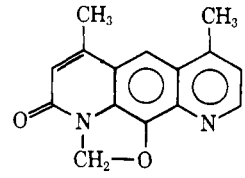
**2**, R = $CH_3$; X = H
**3**, R = X = H
**4**, R = H; X = $NO_2$



**5**



**6**, X = $NO_2$
**7**, X = $NH_2$



**8**

give *o*-methoxyacetoacetanilide ($C_{11}H_{13}NO_3$, mp 83–84°, 85% yield),[6,7] which cyclized in polyphosphoric acid[8] at 100° to **2** ($C_{11}H_{11}NO_2$, mp 187–188°, 80% yield).[6,7] Demethylation of **2** with refluxing 48% hydrobromic acid gave **3** ($C_{10}H_9NO_2$, mp 248–250°, 94% yield),[6,7] which was nitrated[9] in nitric acid–acetic anhydride at 3° to give a mixture of two isomers, from which **4** [$C_{10}H_8N_2O_4$, mp 284° dec; nmr, H-5 at δ 8.34, H-6 at δ 7.77, $J_{56}$ = 9 Hz][6,7] could be isolated in 39% yield. A second isomer, the 5-nitro analog (mp 205–207°, 29% yield; nmr, H-6 at δ 7.82, H-7 at δ 7.40, $J_{67}$ = 8.5 Hz),[6,7] was also isolated. The obvious ortho coupling in the nmr spectra of both isomers served to eliminate the other possible isomers—the 3-nitro and 6-nitro analogs. Assignment of structures to the nitroquinolones isolated was achieved by formation of a benzoxazole derivative from **4**.[10] Reduction of the nitro group in the higher melting isomer followed by treatment with acetic anhydride at 200° in a sealed tube yielded the oxazoloquinolone **5** ($C_{12}H_{10}N_2O_2$, mp 236–238°, 32% yield);[6,7] similar treatment of the lower melting isomer produced no benzoxazole.

Insertion of the methylene bridge into **4** was carried out with methylene bromide and powdered potassium carbonate in dimethylformamide at 100° to afford the bridged quinolone **6** ($C_{11}H_8N_2O_4$, mp 293–295°)[6,7] in 54% yield. Hydrogenation of **6** over platinum oxide gave the aminoquinolone **7** ($C_{11}H_{10}N_2O_2$, mp 224–226°, nearly quantitative yield).[6,7] A modified Doebner–Miller reaction,[11] involving treatment of the hydro-

(1) Paper III: K. L. Rinehart, Jr., G. Leadbetter, R. A. Larson, and R. M. Forbis, *J. Amer. Chem. Soc.*, **92**, 6994 (1970).

(2) H. Naganawa, T. Wakashiro, A. Yagi, S. Kondo, T. Takita, M. Hamada, K. Maeda, and H. Umezawa, *J. Antibiot.*, **23**, 365 (1970).

(3) K. L. Rinehart, Jr., R. A. Larson, R. M. Forbis, and G. Leadbetter, Abstracts, 5th International Symposium on the Chemistry of Natural Products, IUPAC, London, July 1968, p 79.

(4) K. L. Rinehart, Jr., and H. B. Renfroe, *J. Amer. Chem. Soc.*, **83**, 3729 (1961).

(5) A. B. Boese, *Ind. Eng. Chem.*, **32**, 16 (1940).

(6) Microanalyses agree with the molecular formula shown.

(7) Low-resolution mass spectral data agree with the molecular formula shown.

(8) A. K. Mallams and S. S. Israelstam, *J. Org. Chem.*, **29**, 3548 (1964).

(9) R. E. Buckles and M. P. Bellis in "Organic Syntheses," Coll. Vol. IV, N. Rabjohn, Ed., Wiley, New York, N. Y., 1963, p 722.

(10) W. Theilacker, *J. Prakt. Chem.*, [2] **153**, 54 (1939).

(11) K. N. Campbell and I. J. Schaffner, *J. Amer. Chem. Soc.*, **67**, 86 (1945).