Steel, and Weller[11] on the basis of their views of the quenching of fluorescence of aromatic hydrocarbons by quadricyclene. The solvent effects in this example are significantly larger than those observed in the quenching of naphthalene by conjugated dienes.[12]

**Acknowledgment.** This work was supported by the Directorate of Chemical Sciences, Air Force Office of Scientific Research, under Contract No. AF49(638)-1479.

(11) B. S. Solomon, C. Steel, and A. Weller, *Chem. Commun.*, 927 (1969).
(12) G. N. Taylor and G. S. Hammond, manuscript in preparation.
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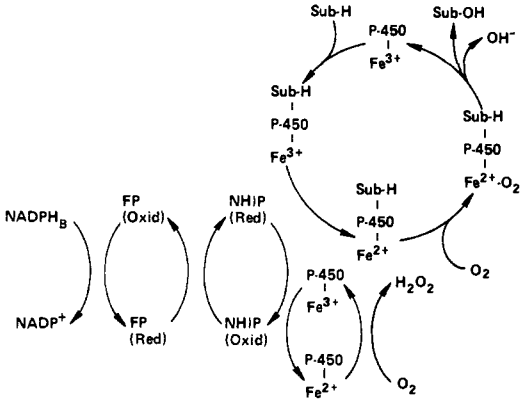
## The Effect of 4,4-Dideuteration of Nicotinamide Adenine Dinucleotide Phosphate on Steroid Hydroxylation

*Sir:*

The 11$\beta$-hydroxylase reaction of adrenal mitochrondria involves the transfer of two reducing equivalents from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to a molecule of oxygen which is in a complex with cytochrome P-450 and deoxycorticosterone (DOC), resulting in the formation of corticosterone (CORT) and $OH^-$. Omura, *et al.*,[1] have purified a flavo protein and a nonheme iron protein, which together form a cytochrome P-450 reductase. Further, they prepared a particle-bound cytochrome P-450, almost devoid of reductase activity, which in the presence of NADPH and reductase was able to hydroxylate DOC to CORT. On the basis of these studies, they postulated that the transfer of reducing equivalents was through the reductase and cytochrome P-450 to the molecular oxygen (Scheme I).

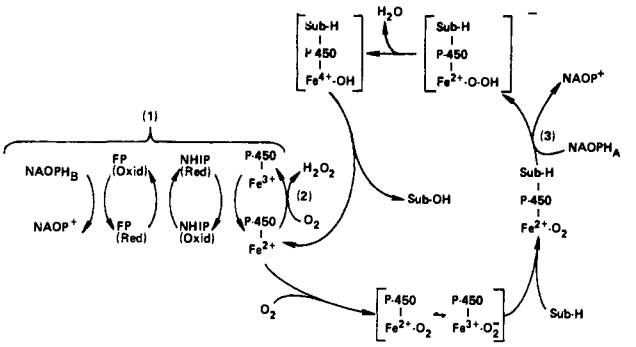
**Scheme I**




(1) T. Omura, E. Sanders, R. W. Estabrook, D. Y. Cooper, and O. Rosenthal, *Arch. Biochem. Biophys.*, **117**, 660 (1966).

Sih, *et al.*,[2,3] have suggested that the reductase merely serves to keep the cytochrome in the reduced or active form. Subsequently, hydrogen is transferred from a second NADPH to form a hydroperoxide which then decomposes to CORT and $OH^-$ (Scheme II).

**Scheme II**




They based their suggestion on the fact that (1) although by manipulations of the proportions of the enzymes, NADH can serve to reduce cytochrome P-450, it does not serve in the hydroxylation reaction; (2) Scheme I should have ping-pong kinetics and yet does not; and (3) the stereochemistry of the dehydrogenation of the dihydronicotinamide ring of NADPH is different for the reduction of the cytochrome and the overall reaction. These observations could be explained on the basis of there being an alternative electron chain and in no way require, as suggested by Sih, *et al.*,[2,3] the existence of an intermediate hydroperoxide. Therefore, the question as to the existence of such a species remains unanswered.

It is well known that the maximal rate of hydroxylations is determined by the rate of reduction of the cytochrome P-450 (reaction 1, Scheme II).[1,4] Yet the actual rate in either scheme will be determined by a competition between the autoxidation of the reduced heme[5] to give an ineffective reaction (reaction 2) and the reduction of the O–O bond to give the hydroxylating species in the effective reaction (reaction 3). These latter two reactions appear to have comparable fluxes of reducing equivalents. Substitution of deuterium for the 4 H's of NADPH (NADP(D)D) would affect neither the rate of reduction nor the rate of autoxidation of the heme, while it would probably slow two–threefold the transhydrogenation involved in the formation of the hydroperoxide and therefore in Scheme II give a two–threefold reduction in the rate of formation of product.[4] No similar isotope effect would be expected in Scheme I. In examining an analogous system, the hydroxylation of ethylmorphine and aniline by hepatic microsomes, I have found that NADP(D)D was nearly as effective in supporting hydroxylation as NADPH.[4] I therefore have examined the 11$\beta$-hydroxylase of adrenal mitochondria to see if it behaves similarly.

NADP(D) and tritiated NADP (NADP(T)) were prepared by the method of San Pietro[6] and purified by

(2) C. J. Sih, Y. Y. Tsong, and B. Stein, *J. Amer. Chem. Soc.*, **90**, 5300 (1968).
(3) C. J. Sih, *Science*, **163**, 1297 (1970).
(4) J. L. Holtzman, *Biochemistry*, **9**, 995 (1970).
(5) M. L. Sweat, R. B. Young, and M. J. Bryson, *Arch. Biochem. Biophys.*, **130**, 66 (1969).
(6) A. San Pietro, *J. Biol. Chem.*, **217**, 579 (1955).

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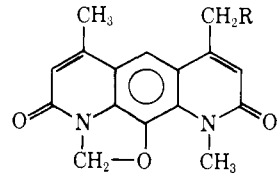
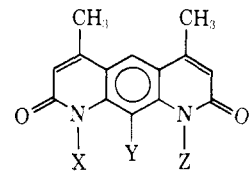
## Nybomycin. III. A Revised Structure[1,2]

*Sir:*

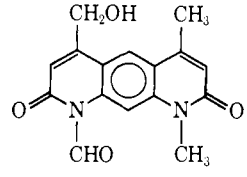
The antibiotic nybomycin was isolated some years ago in two laboratories from streptomyces cultures;[3,4] it is quite active against Gram-positive bacteria, but is insoluble. Deoxynybomycin, originally described by us as a degradation product of nybomycin,[5] was very recently reported by Umezawa, *et 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.



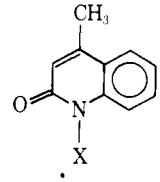
**1**, R = OH
**2**, R = H
**10**, R = -OCO(CH$_2$)$_2$CH$_3$
**12**, R = D
**13**, R = -OCOCF$_3$



**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**, $C_{15}H_{13}NO_3$, 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**, $C_{20}H_{22}N_2O_5$, 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.