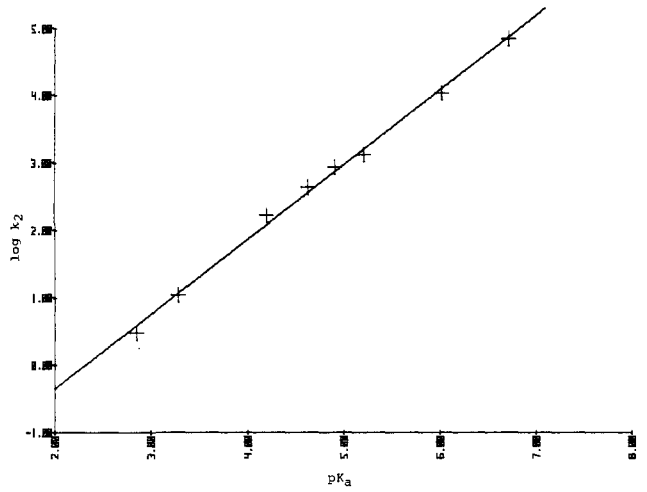

Figure 1. Brönsted plot of log k_2 vs. pK_a for the base-catalyzed proton exchange of *N,N*-dimethylcyclohexylamine hydrochloride in chloroform at 25°. Rate constants are in M^{-1} s^{-1} and pK_a values are taken from Table I.

set at an rf field of 0.1 mG, sweep width of 54 Hz, sweep time of 250 s, and filter band width of 20 Hz. An uncertainty of 2.5 eu in our ΔS^* values reflects a 10-15% error in rate constants obtained over a 40-45° temperature interval.

The rate constants in Table I cover a range of 2×10^4 and correlate closely with the basicity of the catalysts. A Brönsted plot, with a slope $\beta = 1.1$ (Figure 1), points to a well-formed catalyst/proton bond in the transition state. Since this Brönsted plot utilizes aqueous pK_a values (not pK_a's in chloroform), the value of β is meaningful only under the assumption that the pK_a shift from water to chloroform is constant for all pyridine bases. However, a $\rho = -6.4$ for a Hammett plot based on 3- and 4-substituted pyridines (not shown) confirms that proton transfer in eq 1 is virtually complete in the transition state.[6] In the light of a nearly identical charge content of the ground state and transition state, how can the large negative ΔS^* values be explained? One possibility is that the solvation requirements for protonated aliphatic amine and protonated pyridine differ substantially despite their identical charges. The validity of this rationale is demonstrated from the ΔS^* values for the substituted pyridines (Table I) which gravitate from −28 to −40 eu as the basicity of the catalysts decreases. The poorer the base, the greater the solvation needs of the corresponding conjugate acid, and the more negative the ΔS^*. We add parenthetically that the marked dependence of ΔS^* on the nature of the positive charge demands great caution when using ΔS^* as a criterion for the creation of charge.

Solvation differences between the reactant and product cations did not seem to be the sole explanation for the large negative entropies because ΔS^* approximates −30 eu even for the more basic catalysts. We therefore investigated whether or not the *anion* of I might also influence the proton transfer. The relative rate of pyridine-catalyzed proton exchange of I in chloroform increases from 1.0 to 7.6 to 52 as the counterion of I is changed from chloride to bromide to benzenesulfonate. Corresponding entropies are −31, −23, and −20 eu. These results can be best explained by an ion-pair dissociation (eq 2)[11] which precedes the rate-determining proton abstraction and which of course contributes to the observed ΔS^*.

$$R_3NH^+Cl^- \rightleftharpoons Cl^- + R_3NH^+ \xrightarrow{C_6H_5N} R_3N + C_6H_5NH^+ \quad (2)$$

Since disengaging X^- from an ion-pair immobilizes solvent according to the charge density on X^-, the chloride salt would be expected to have the most negative ΔS^*. Equation 2 is also consistent with another observation, namely that ΔS^* for the pyridine-catalyzed proton exchange of I (chloride salt) in pure ethanol is only −18 eu. Dissociation of an anion from a "loose" ion-pair within a protic solvent demands less *additional* solvent stabilization of the anion. In summary, the exceptionally negative entropy of activation of eq 1 stems from several factors: the bimolecularity of the reaction; solvation differences between the donor and acceptor amines; freezing of solvent around the anion following ion-pair dissociation.

Interestingly, the severely hindered base 2,6-di-*tert*-butylpyridine displays normal reactivity (its k_2 lies on the Brönsted line, not below it).[12] No doubt steric interactions between two bulky amines sharing a proton are reduced by the late transition state for eq 1. Freedom of k_2 and ΔS^* from steric effects also suggests that the two nitrogens and the proton in eq 1 prefer a rather inflexible linear orientation in the transition state.[13,14]

Acknowledgment. This work was supported in part by grants from the National Science Foundation (Grant No. CHE-7402279) and by the National Institutes of Health (Grant No. GM-20336 and GM-21457). We thank Professors Marjorie Caserio and Robert Taft for their valuable input.

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(15) Recipient of a Camille and Henry Dreyfus Foundation Teacher-Scholar Grant and a National Institutes of Health Research Career Development Award.

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Nybomycin. 8. Biosynthetic Origin of the Central Ring Carbons Studied by ^{13}C-Labeled Substrates[1,2]

Sir:

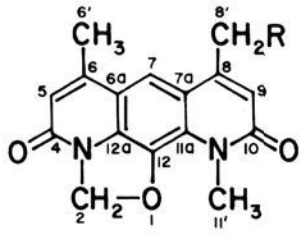
The antibiotic nybomycin (**1**) possesses two structural features of biosynthetic interest, a fused pyridoquinolone ring system and an angularly fused oxazoline ring. Both features have not been reported elsewhere in nature except for the naturally occurring deoxynybomycin (**2**).[3] We recently established by use of ^{14}C- and ^{13}C-labeled precursors that the single-carbon units C-11′ and C-2 (N-CH_3 and N-CH_2-O, respectively) are derived from methionine, while the exterior carbons of the pyridone rings (C-4 to C-6, C-6′, C-8 to C-10, C-8′) arise from acetate.[4] The ^{13}C-labeled acetate feeding also showed that the central ring carbons are not derived from this source and, thus, eliminated the possibility of the aromatic system's arising from a phloroglucinol-type pathway.[5] We now present evidence which supports the intermediacy of a shiki-

Table I. ^{13}C NMR Peak Assignments and ^{13}C-Enrichment Data for Nybomycin *n*-Butyrate (**3**)

Carbon atom	Absorption, ppm	I_e/I_u[a] D-[6-^{13}C]-Glucose	I_e/I_u[a] Sodium [2-^{13}C]-pyruvate
C-2	85.9	1.8	5.6
C-4	158.1	1.1	12.8[b]
C-5	121.5	1.6	1.0
C-6	147.1	1.0	11.6[b]
C-6′	17.8	1.8	0.8
C-6a	113.4	1.0	9.8[b]
C-7	112.0	2.5	1.0
C-7a	117.4	1.2	9.4[b]
C-8	143.7	1.2	11.4[b]
C-8′	61.9	1.6	0.9
C-9	118.6	1.9	1.3
C-10	161.3	1.0	11.2[b]
C-11a	125.6	1.5	1.3
C-11′	32.6	1.6	5.7
C-12	135.5	1.0	2.3[b]
C-12a	132.2	1.5	1.2
C-1″	172.7	1.1	0.8
C-2″	36.0	1.0	1.1
C-3″	18.4	1.1	1.0
C-4″	13.7	0.9	1.0

[a] I_e and I_u refer to intensities in spectra (obtained under identical instrumental conditions) of enriched and unenriched nybomycin *n*-butyrate, respectively, with all peaks normalized to the average peak intensity of C-1″, C-2″, C-3″, and C-4″ of **3**. [b] Minimum values.[15]

mate-type pathway in formation of the central aromatic nucleus of nybomycin.



1, R=OH

2, R=H

3, R=$O_2CCH_2CH_2CH_3$

In a previous publication the ^{13}C NMR spectrum of nybomycin *n*-butyrate (**3**) was tentatively assigned with the help of standard chemical-shift data, off-resonance proton decoupling, ^{13}C–^{1}H splitting patterns, and consideration of the effects manifest in the proton magnetic resonance spectrum of **3**.[4b] More recently, detailed examination of related model compounds and of ^{15}N-enriched analogues has allowed definitive assignment of all of the carbon absorptions (Table I). These assignments have been discussed in detail in a separate publication,[6] but we can note here that a novel use of ^{13}C–^{15}N coupling constants observed for biosynthetically ^{15}N-enriched **3** was instrumental in unambiguous assignment of three of the aromatic carbon resonances undeterminable by the model study. None of these modifications, however, has altered the biosynthetic results described previously.[4]

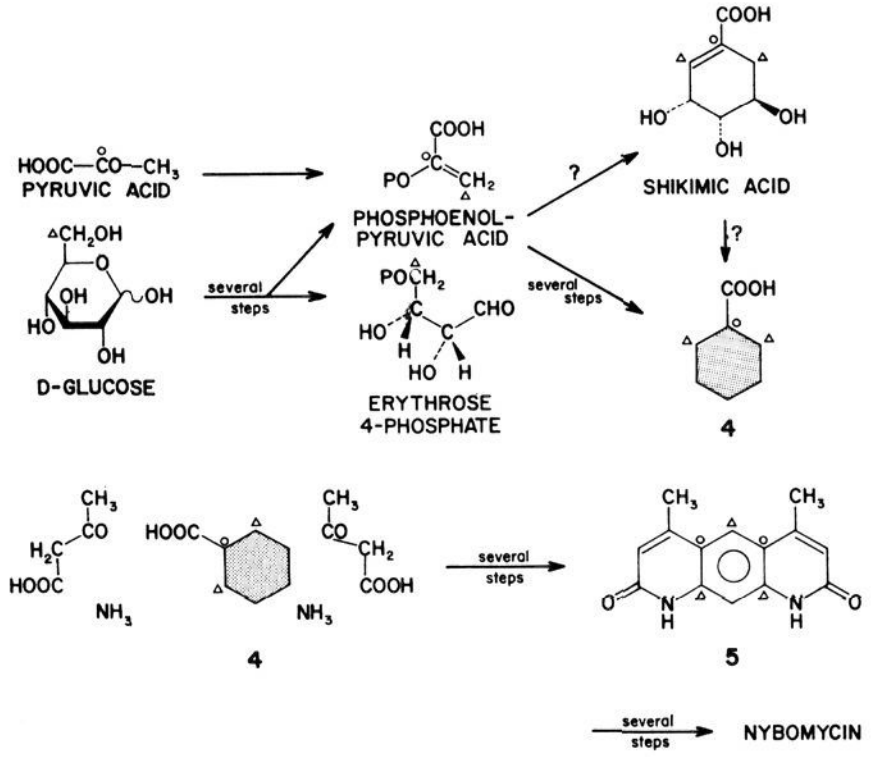


Figure 1. Proposed scheme for nybomycin biosynthesis, showing labeling patterns for central ring carbons from D-[6-^{13}C]glucose (△) and sodium [2-^{13}]pyruvate (○). The substitution pattern and degree of unsaturation are unspecified in intermediate **4**.

A shikimate-type intermediate was considered as a possible source of the central ring carbons in view of the known biochemical origin of aromatic amino acids[7] and other aromatic natural products.[8] To assess the likelihood of a shikimate pathway, D-[6-^{14}C]glucose, sodium [2-^{14}C]pyruvate, and D-[4-^{14}C]erythrose[9] were administered and shown to be well incorporated (Table II). To locate the labeled carbons D-[6-^{13}C]glucose (63 atom %, 0.75 g in 900 ml)[10] was administered on the eighth day to *Streptomyces sp.* D-57, growing on a modified starch–nitrate medium.[11] The crude antibiotic was isolated after an additional 10 days growth of the organism, then converted to the more soluble *n*-butyrate (**3**) and purified. Isotope ratio mass spectral analysis showed 92.7% unlabeled butyrate, 4.5% monolabeled, 2.0% dilabeled, and 0.6% trilabeled. The ^{13}C NMR spectrum of labeled **3** indicated significant enrichment ($I_e/I_u \geq 1.5$) in nine carbons (Table I), including three of the aromatic carbons (C-7, C-11a, and C-12a).

Table II. Incorporation of Labeled Precursors into Nybomycin

	D-[4-^{14}C]Erythrose	D-Glucose [6-^{14}C]	D-Glucose [6-^{13}C]	Sodium pyruvate [2-^{14}C]	Sodium pyruvate [2-^{13}C]
Precursor added					
Amount	1.1 mg	1.8 mg	0.75 g	0.31 mg	1.0 g
Label	5.0 mCi/mmol	5.11 mCi/mmol	63% ^{13}C	9.89 mCi/mmol	90% ^{13}C
Nybomycin isolated					
Amount[a]	68 mg	52 mg	80 mg	76 mg	78 mg
Label	3.16 μCi/mmol	4.94 μCi/mmol	1.2% ^{13}C[b]	5.17 μCi/mmol	21% ^{13}C[b]
% incorporation	1.6	1.7	1.1[c]	4.7	4.4[d]
Dilution	1582	1034	52[b]	1913	4.3[b]

[a] Based on amount of nybomycin present in mycelia; determined by bioassay. [b] Based on percent label at each labeled carbon estimated from mass spectra of nybomycin butyrate (**3**). [c] Based on nine labeled carbons (each 1.2%) per nybomycin. [d] Based on eight heavily labeled carbons (each 21%) per nybomycin.

The labeling of alternate aromatic carbons would be in accord with a phloroglucinol pathway, but this was eliminated in earlier studies.[4] This labeling would also be in accord with a shikimate-type pathway provided, at some stage in the biosynthesis, a symmetrical intermediate is produced. Thus, C-1 of shikimate or a related precursor would become C-6a and C-7a of a nybomycin precursor such as **5** (cf. Figure 1). Furthermore, since C-6 of glucose is distributed between C-2 and C-6 of shikimate in about equal amounts,[12] enrichments at C-7, C-11a, and C-12a of nybomycin should be in the approximate ratio 2:1:1; this is roughly observed (3:1:1) in the enrichment factors ($I_e/I_u - 1$) from peak intensity data (Table I). Since determination of carbon peak intensities is less than quantitative[13] the data at least qualitatively support the proposed route.

Additional evidence for a shikimate-type biosynthetic pathway was obtained by administering sodium [2-^{13}C]pyruvate (90 atom %, 1.0 g in 900 ml)[14] to producing cultures of *Streptomyces sp.* D-57. The nybomycin butyrate derived from this feeding was highly labeled (Table II); the ^{13}C NMR spectrum indicated very high enrichment (>5 times natural abundance) in eight carbon atoms (Table I).[15] Only two of the central aromatic carbons of **3**, C-6a and C-7a, were highly enriched and in about equal amounts. As a consequence of the high enrichment levels, resonances at C-6a and C-7a were each split into a doublet of triplets ($^1J_{CC}$ = 54 Hz, $^3J_{CC}$ = 4 Hz) by virtue of direct coupling ($^1J_{CC}$) to C-6 and C-8, respectively, and long range coupling ($^3J_{CC}$) to two other highly enriched centers (C-4 and C-8 for C-6a; C-6 and C-10 for C-7a). Magnitudes of J_{CC} and coupling patterns observed ($^3J_{CC} > {}^2J_{CC}$) are in accord with established couplings for related aromatic systems.[16]

The above labeling pattern is completely consistent with the proposed biosynthetic scheme (Figure 1). The high level of incorporation at C-6a and C-7a follows directly from conversion of C-2 of pyruvate via phosphoenolpyruvate to the carboxyl bearing carbon (C-1) of shikimate[12] or a related, possibly symmetrical, precursor **4**.[17] Although labeling patterns from both feeding experiments support the intermediacy of a symmetrical intermediate in nybomycin biosynthesis they do not establish at what stage the proposed intermediate becomes symmetrical, i.e., whether the symmetrical intermediate is monocyclic (**4**) or tricyclic (e.g., **5**). Experiments to characterize intermediates are in progress.

Finally, labeling of carbons outside the central ring in both precursor feeding experiments may be rationalized via known biochemical pathways existing in most microorganisms[20] and from results of previous biosynthetic studies on nybomycin. Thus, D-[6-^{13}C]glucose and sodium [2-^{13}C]pyruvate are metabolized to acetate (labeled at C-2 and C-1, respectively), which supplies the outer carbons of each pyridone ring. Both precursors label the anticipated carbons (Table II). Enhancements at C-2 and C-11′ arise from channeling the appropriate labeled carbons of glucose and pyruvate into the "one carbon" metabolic pool, which is readily accomplished through serine metabolism.[21]

Acknowledgment. This work was supported by Public Health Service Grants AI 01278 and AI 04769 from the National Institute of Allergy and Infectious Diseases. The ^{13}C Fourier transform NMR spectra and mass spectra were obtained on instruments purchased, in part, with grants from the National Science Foundation, the National Cancer Institute (CA 11388), and the National Institute of General Medical Sciences (GM 16864). We thank Mr. Stephen Silber for the ^{13}C NMR spectra, Mr. Joseph Wrona for the isotope ratio mass spectra, The Upjohn Company for a soil stock of *Streptomyces sp.* D-57 and a sample of nybomycin, and Dr. Masao Taniguchi for a sample of D-[6-^{13}C]glucose.

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2,6-Substituted Homotropilidenes. Influence of Substituents on Valence Topomerization

Sir:

The Cope rearrangement which involves a bond breaking of one σ bond and the formation of another is one of the best studied reactions.[1] Nevertheless, different opinions exist about