piperylene inhibited the decomposition of BPO as well as the CIDNP phenomena. This finding is also consistent with previous observations that decomposition of BPO and hence CIDNP is not induced by use of sensitizers with E_T lower than 55 kcal/mol.[8,9] The same CIDNP spectra were also observed in oxygen-saturated chloroform-*d*, although the rate of decomposition of TMD was four times slower than in degassed solution (so CIDNP spectra were seen over a longer period) presumably on account of inhibition of chain decomposition of TMD previously observed.[10]

The opposite polarization phases in direct and photosensitized decomposition of BPO illustrate the effect of singlet vs. triplet radical pair precursors on the product. We have also encountered in the course of this work an illustration of the effect of the magnetic field on the phase of the CIDNP signal. Chlorobenzene produced by the acetophenone-photosensitized decomposition of benzoyl peroxide in CCl_4 at 30° *outside* the NMR spectrometer showed a strong emission signal, in contrast to the enhanced absorption noted by Kaptein et al.[6] under identical conditions *inside* the magnetic field. Such field dependencies are well known.[7] A striking, and yet unexplained, feature of this experiment is the persistence of our emission signal for 1 min after cessation of the illumination, whereas in the photodecomposition inside the magnetic field[6] the signal disappeared immediately on turning off the light.[11]

Dependence of the CIDNP phase on the nature of the radical pair is strikingly illustrated by the comparison of the results with benzoyl peroxide and those with *tert*-butyl perbenzoate. The chlorobenzene from the TMD-induced decomposition of *tert*-butyl perbenzoate in carbon tetrachloride gives a weak emission signal, under the same conditions that produced enhanced absorption from benzoyl peroxide. In both cases the signal reaches its maximum intensity 45–50 sec after insertion of the sample into the probe at 87°. From what is known about the *g* factors of phenyl (2.0020)[12] and *tert*-butoxy (2.009)[13] radicals, a phenyl-*tert*-butoxy radical pair would not be expected to produce this reversal of CIDNP phase. Perhaps in this case the initial pair (t-BuO··OOCC$_6$H$_5$) determines the polarization, which is impossible from the corresponding symmetrical pair from benzoyl peroxide.

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(11) One may speculate that transfer of our sample from the light into the NMR probe dissipated any heat buildup from the irradiation while a temperature rise in the irradiated sample of ref 6 might have allowed a later slow thermal decomposition of the BPO, the emission phase of whose chlorobenzene CIDNP counteracted the residual absorption.
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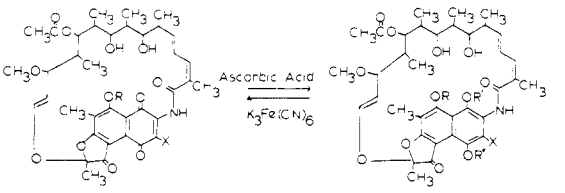
Electronegative Groups at C-3 of Rifamycin S Enhance Its Activity toward DNA-Dependent RNA Polymerase

Sir:

Rifamycin S (**1a**), a fermentation product of *Streptomyces mediterranei*,[1] is a potent inhibitor of DNA-dependent RNA polymerase (RNAP) of *E. coli* and other prokaryotes.[2] Rifamycin derivatives have also been observed to inhibit RNA-dependent DNA polymerase, but substantially higher concentrations are required in this case.[3] Little is known concerning the details of the remarkably tight interaction between the ansamycins and RNAP although it appears that covalent bond formation is not involved.[4] The potentially promising antiviral properties of these antibiotics[5] has prompted us to investigate the details of this interaction. We report here that inhibition of RNAP by 3-substituted rifamycins is (with one exception) increased by electron attracting and decreased by electron donating substituents, and that this, in all likelihood arises from a variation in k_{assoc} for the formation of the known 1:1 RNAP:rifamycin complex.[6]

Rifamycin derivatives **1f–j** were prepared by reaction of rifamycin S with the appropriate nucleophile. Halogen derivatives **1d** and **1e** were prepared by halogenation of rifamycin SV. Derivative **1c** was prepared by halide exchange of **1d**.[7]



X		R
H	1a	H, RIFAMYCIN S
CH=N-(4-CH$_3$-piperazin-1-yl)	1b	H
Cl	1c	H
Br	1d	H
I	1e	H
CN	1f	H
NH-(N-O)	1g	H
NH_2	1h	H
$NH(CH_2)_4$-(phenyl)	1i	H
$SC(CH_3)_3$	1j	H
H	1k	CH_3

	R	R′	R″
2a	H	H	H, RIFAMYCIN SV
2b	H	H	H, RIFAMPICIN
2c	H	H	H
2d	H	H	H
2e	H	H	H
2f	H	H	H
2i	H	H	H

DNA-dependent RNA polymerase was isolated from *E. coli* K-12 using a modification of Burgess' procedure,[8] with final purification accomplished by means of a DNA-affinity column.[9] The purity of the isolated enzyme was determined by SDS gel electrophoresis to be at least 95%.[10] In vitro assays preincubated 26 μg of RNAP and varying amounts of antibiotic at 4°C in a 230 μl solution containing a final concentration of 40 m*M* Tris·HCl (pH 7.9), 10 m*M* $MgSO_4$, 150 m*M* KCl, 0.5 mg/ml bovine serum albumin, 0.15 m*M* UTP, GTP, CTP, and ^{14}C-ATP (2mCi/mmol), and 0.1

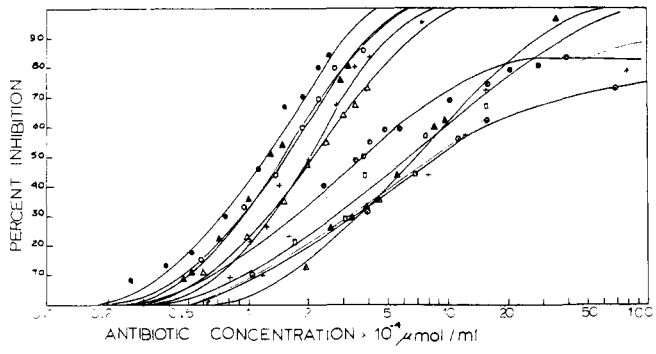


Figure 1. Inhibition curves for rifamycin S and its 3-substituted derivatives. The data for all the tested rifamycin S derivatives (●, **1e**; ▲, **1b**; ○, **1c**; +, **1d**; △, **1j**; ◐, **1a**; □, **1h**; ▲, **1f**; ⊙, **1g** and *, **1i**) are an average of three-five sets of enzyme assays at constant enzyme concentration (26 μg). The range of values at each point being ±3% in the area between 20 and 80% inhibition. The 3-substituted rifamycin SV derivatives were obtained by ascorbic acid reduction of the corresponding 3-substituted rifamycin S derivative. The inhibition data on the 3-substituted rifamycin SV compounds were obtained in the manner described for the 3-substituted rifamycin S derivatives but are omitted from this figure.

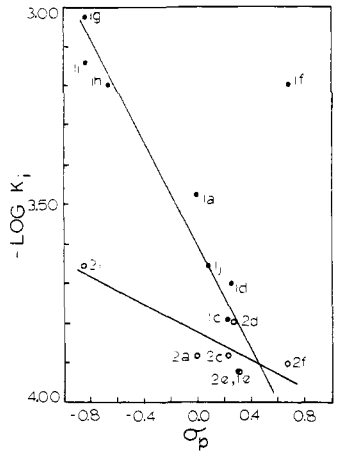


Figure 2. Hammett plot of $-\log k_i$ vs. σ_p. The closed circles (●) correspond to the respective 3-substituted rifamycin S derivatives (**1a–1i**), $\rho(\log k_i) = -0.71$, and the open circles (○) correspond to the 3-substituted rifamycin SV derivatives (**2a–2i**), $\rho(\log k_i) = -0.24$.

m*M* EDTA. RNA synthesis was initiated by addition of 30 μg of calf thymus DNA (1.5 mg/ml), followed by incubation for 10 min at 37°C. The reaction was quenched by addition of 20 μl of 10 m*M* ATP in 0.2 *M* EDTA (pH 7) at 4°C, and three 75-μl aliquots from each tube were spotted on three DE-81 filter papers, washed with 5% disodium phosphate, and counted.[11]

Plots of activity vs. antibiotic concentration at fixed RNAP concentration are shown in Figure 1. Inhibition curves did not vary with different enzyme isolates, antibiotic preparations, or assay stock solutions. Inhibition constants (k_i), the molar concentration of enzyme affording 50% inhibition of enzyme activity,[12] were calculated from Figure 1 and by a probit analysis of the data.[13] A linear correlation (cyano derivative excepted)[14] between log k_i and σ para is observed (Figure 2), but no correlation was found between k_i and σ meta, covalent radii of the atom attached to the rifamycin ring, or rather substantial changes in the conformation of the ansa bridge evinced by the proton NMR spectra of the antibiotics.[15] As is apparent from the antibiotic concentration necessary for 50% inhibition of RNAP (Figure 1) there is a substantial dependence of activity on substituent.

The simplest explanation of these data is that one is observing a dependence of the rifamycin-RNAP association constant (k_{assoc}) on structure. This explanation is supported by the following experiments. First, an enzyme sample 85% inhibited was prepared by incubating 12 μg of RNAP with 7 mol equiv of 3-nitroxide rifamycin S (**1g**) for 10 min at 4°C. If this sample is then diluted into assay buffer containing DNA, RNA synthesis is observed (Table I). In the case of rifampicin (**1b**) one observes no synthesis on dilution, this being consistent with the known slow dissociation of the RNAP-rifampicin complex.[4] The same effect is seen when excess **1g** and **1i** are removed with activated charcoal.[16] Activity is not restored by this technique in the case of rifampicin (**1b**) and rifamycin SV (**2a**) (Table II). Third, a kinetic analysis[17] using CTP as a substrate in the normal assay buffer indicated that for compound **1g** hyperbolic noncompetitive inhibition was occurring as compared to the linear noncompetitive inhibition demonstrated by rifampicin. These results are consistent with a small k_{assoc} for the 3-nitroxide rifamycin S complex and, in case of high 3-nitroxide rifamycin S concentration, formation of an unstable substrate-enzyme-inhibitor complex which regenerates viable enzyme.

At the pH (7.9) of the assay all of the derivatives are demonstrably in the monoionic form. The total inactivity of the methyl ether (**1k**)[18] not withstanding, it seems unlikely that one is observing a crucial substituent effect on the pK_a of the phenolic hydroxyl. We suggest that the effect of introducing electronegative substituents is simply explained by the napthoquinone ring being involved in a donor-acceptor π-complex interaction, the k_{assoc} then being a function of the π-complex lifetime. The interaction with some (possibly a tyrosine) residue may occur by insertion of the residue into the hole formed by the ansa bridge and the napthoquinone ring.[19]

Table I. Dilution Experiment for the RNA polymerase, and RNA Polymerase Complex with the Nitroxide, **1g**, Phenylbutylamine, **1i**, and Rifampicin, **1b**

		% inhibition[b]		
Dilution	RNA polymerase standard[a]	RNA polymerase **1g**	RNA polymerase **1i**	RNA polymerase **1b**
0	1.1	87	95	98
2	1.2	84	89	98
2.5	1.2	79	87	--
5	1.2	66	81	98
10	1.1	60	79	--

[a] Expressed as mμmol of AMP incorporated (^{14}C-ATP, 0.15 m*M*, 25 mCi/mmol). [b] Per cent decrease in synthesis by the RNA polymerase standard.

Table II. Charcoal Treatment of Rifamycin–RNAP Complexes

	Specific activity obsd (mμ mol of AMP incorporated)/(μg of protein)[b] × 10^{-4}				
No. of charcoal[a] treatments	RNAP	(**1g**)–RNAP	(**1i**)–RNAP	(**1b**)–RNAP	(**1a**)–RNAP
0	7.6	1.6	1.1	0.24	0.28
1	7.1	1.9	1.2	0.34	0.35
2	7.9	3.5	2.5	0.75	0.71

[a] Acid washed Darco 60 washed with water until neutral and the fines removed was used to absorb excess rifamycin derivatives. [b] In a total volume of 1.0 ml, 50 μg of RNAP, 300 μg of Bovine serum albumin, and 100 mol equiv of the respective rifamycin derivative (except in the case of the protein standard) were incubated at 4°C for 10 min. After charcoal treatment the samples were centrifuged for 2 min at 4°C and aliquots removed for Lowry protein assay and radioactive assay. The supernatant was removed and treated with fresh charcoal and the process repeated.

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(19) We would like to thank Ms. Andrea Stencil and Ms. Louise Hellwig for synthesizing the 3-bromorifamycin S and the 8-*O*-methyl ether rifamycin S.

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Novel Polar Photochemical Additions of Acetic Acid to Phenylallenes

Sir:

Photochemical polar addition of a protic solvent to a multiple bond has been one of the most intriguing research subjects.[1] The addition reaction may be divided into two categories (A and B) on the basis of the type of the excited state involved. (A) The reactions of medium size cycloalkenes or strained cycloalkynes seem to proceed through both the triplet excited state[1a-l] and the singlet excited state, although the reaction via the singlet excited state is rather exceptional.[1c] (B) The reactions of acyclic alkenes or acyclic alkynes appear to proceed through the singlet excited state.[1l-w] However, regardless of the multiplicity of the excited state, photochemical polar addition to a multiple bond unconjugated to an electron-withdrawing group occurs generally in the Markownikoff fashion.[2]

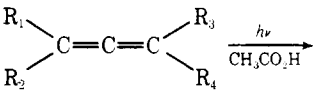
In the present study, we wish to report the first observation of the photochemical polar addition of acetic acid to phenylallenes.

Table I. The Product of the Photochemical Addition of Acetic Acid to Allenes

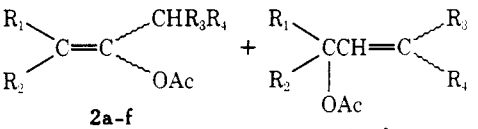
Allene	Irradiation period (hr)	Product[a] (%)	Recovered[a] allene (%)
1a	70	**3a** (3)	**1a** (75)
1b	72	**2b** (2)	**1b** (74)
1c	48	**2c** (65)	**1c** (31)
1d	48	**2d** (45)	**1d** (47)
1e	48	**2e** (40)	**1e** (51)
1f	96	Trace	**1f** (nq)[b]

[a] The percentage is based on the amount of the allene initially used. [b] Nearly quantitative.

A solution of an allene (0.5 *M*) in acetic acid was irradiated with a 15-W low-pressure mercury lamp (mainly 254 nm) to give an enol acetate or an allyl acetate (Table I).[3] The allenes **1a–f** were stable under conditions identical with those of the irradiations except for the absence of light.



1a-f



2a-f **3a-f**

a, R_1, R_3 = $(CH_2)_6$; $R_2 = R_4 = H$
b, $R_1 = n\text{-}C_6H_{13}$; $R_2 = R_3 = R_4 = H$
c, R_1 = Ph; R_2 = Me; $R_3 = R_4 = H$
d, R_1 = Ph; $R_2 = R_3$ = Me; $R_4 = H$
e, $R_1 = m\text{-}$MeOPh; R_2 = Me; $R_3 = R_4 = H$
f, $R_1 = m\text{-}CF_3$Ph; R_2 = Me; $R_3 = R_4 = H$

Although about 90% of the photochemical reaction of **1c** (0.5 *M*) could be quenched by 1,3-pentadiene (0.5 *M*), about 10% of the reaction remained even though the concentration of the quencher was increased above 0.5 *M*.[4] Furthermore, sensitization of the reaction by xanthone was observed when the reaction mixture was irradiated with uv light (>300 nm) which is not absorbed by the allene **1c**. These facts indicate that the reaction of **1c** seems to proceed mainly through the excited triplet state.

In the reactions of the aliphatic allenes **1a** and **1b**,[5] the products, **3a** and **2b**, were the same as those obtained from the nonphotochemical reaction of the corresponding allenes with acetic acid in the presence of a trace amount of sulfuric acid. In contrast, the results of the photochemical reactions of the phenylallenes **1c**, **1d**, or **1e** were quite different from those of the known protonic acid-catalyzed reactions[6] (Scheme I), in which the initial attack of a proton was reported to occur at the central carbon (β). On the other hand, in the photochemical addition to **1c–e**, proton attack is observed on the terminal carbon (γ). It is interesting to note that although styrene type olefins are well known to be far more reactive than simple olefins, the nonconjugated olefinic part (C_β=C_γ) was the exclusive reaction site in the present reactions. The marked difference between the yields of the reactions of **1a–b** and those of **1c–e** clearly indicates the remarkable effect of the phenyl group on the reaction.[5] Furthermore, metasubstitution of a strongly electron-withdrawing group (CF_3) on the phenyl ring retarded dramatically the photochemical reaction.

From these observations, it might be suggested that a polarized excited state such as **4** or **5** is involved in the reaction. The tentative species **4** or **5** could rationalize the regioselective addition of acetic acid and the inertness of **1f**.