purified by paper chromatography. The adenosine-U-C^{14} was eluted from the paper, sterilized, and added to cultures of *C. militaris*[6] that were rapidly synthesizing cordycepin. The formate-C^{14} solution was added in a similar manner. Five to seven days after the addition of formate or adenosine, the cordycepin was isolated, purified, and crystallized to constant specific activity.[7] The radioactivity added to the flasks and the incorporation into cordycepin are shown in Table I.

TABLE I

INCORPORATION OF RADIOACTIVE COMPOUNDS INTO CORDYCEPIN[a]

Expt. no.	Compound[b]	Added mg.	Added μc.	Added μc./mmole	Cordycepin μc./mmole
1	Adenosine-U-C^{14}	9.0	24	713	18.0
2	Adenosine-U-C^{14}	1.5	6.0	1070	3.56
3	Adenosine-U-C^{14} plus D-ribose	1.5	6.0	1070	3.58
4	Formate-C^{14}	68.0	50.0	50.0	0.60

[a] Samples were counted in a Packard Tri-Carb scintillation spectrometer in dioxane–naphthalene scintillation solution. [b] Carbon source: expt. 1, 2, and 3, acetate; expt. 4, glucose.

Adenosine-U-C^{14} and formate-C^{14} were both incorporated into cordycepin. The similar specific activities of cordycepin in expt. 2 and 3 tend to rule out cleavage of the carbon–nitrogen glycoside bond of adenosine. Further proof of the direct conversion of adenosine to cordycepin is shown below.

To determine the distribution of radioactivity in cordycepin from the formate, adenosine-U-C^{14}, and adenosine-U-C^{14} plus D-ribose experiments, the cordycepin and adenosine-U-C^{14} were hydrolyzed, concentrated, and chromatographed on paper. The chromatograms were developed in ammonia–water (pH 10.0). One radioactive spot was observed for the adenine, ribose, or cordycepose, and these were in agreement with the R_f values of authentic adenine, ribose, and cordycepose obtained by hydrolysis of nonradioactive nucleosides. These spots were eluted and the radioactivity measured. The distribution of C^{14} in cordycepin and adenosine is shown in Table II.

TABLE II

THE DISTRIBUTION OF C^{14} IN ADENOSINE AND IN CORDYCEPIN FROM ADENOSINE-U-C^{14} AND FORMATE-C^{14}

Expt. no.	Substrate	Compound hydrolyzed	% C^{14} in Adenine	% C^{14} in Ribose	% C^{14} in Cordycepose
		Adenosine	36.6	63.4	
1	Adenosine-U-C^{14}	Cordycepin	40.6		59.4
2	Adenosine-U-C^{14}	Cordycepin	33.5		66.5
3	Adenosine-U-C^{14} plus D-ribose	Cordycepin	37.0		63.0
4	Formate-C^{14}	Cordycepin	98.0		2.0

Although formate-C^{14} (expt. 4) was incorporated into cordycepin, 98.0% of the radioactivity resided in the adenine. This indicates that the formate entered the C-1 pool of the fungus, but the low incorporation into cordycepose suggests that this pentose does not arise by a C-1, C-4 condensation. If cordycepin arises from the direct conversion of adenosine, then the distribution of radioactivity between adenine and the sugar should be the same for the isolated cordycepin as for the adenosine regardless of the structure of cordycepin (I or II). Hydrolysis of the added adenosine-U-C^{14} resulted in 36.6% of the C^{14} in adenine and 63.4% in ribose (Table II). Similarly, distribution of C^{14} in cordycepin from expt. 1 and 2 compares with that of adenosine. The distribution of radioactivity in cordycepin was not affected when 10 mg. of ribose was added along with adenosine-U-C^{14} (expt. 3). An average of 98.8% (expt. 1, 2, and 3) of the radioactivity of the ribose from adenosine was found in the cordycepose. The ribose moiety from the adenosine-U-C^{14} was isolated, converted to the osazone,[8] and degraded[9] in order to determine the distribution of radioactivity in the sugar. The distribution of C^{14} obtained from the ribose of adenosine-U-C^{14} is given in Table III.

TABLE III

DISTRIBUTION OF C^{14} FROM RIBOSE-U-C^{14}

Derivative	Carbon atoms	C.p.m./mmole[a]	% C^{14} Exptl.	% C^{14} Theoretical
Ribosazone	1,2,3,4,5	19,550	100	100
Mesoxaldehyde 1,2-bisphenylosazone	1,2,3	11,394	58.4	60
Formaldimedon	5	3,636	18.6	20
Formic acid	4	[b]		20

[a] Crystallized to constant specific activity. [b] Not analyzed.

These findings rule out the hydrolysis of adenosine prior to the formation of cordycepin and *demonstrate that none of the carbon atoms of ribose is lost.*

Studies are in progress to define the reaction sequence and to determine if the biosynthesis of this deoxynucleoside proceeds in a manner similar to that of deoxyribonucleotides.[10]

(6) Kindly supplied by Dr. A. J. Guarino, Dept. of Biochemistry, Woman's Medical College of Pennsylvania, Phila., Pa.

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On the Enantiomorphism of Organic Peroxy Compounds in the Crystalline State

Sir:

Because of the dihedral angle of the peroxy group, a molecule of an organic peroxy compound is not identical with its mirror image. In solution, the low barrier[1] to rotation about the peroxy link results in frequent interconversion between the mirror-related configurations, so that although nonzero dipole moments have been reported,[2] D,L isomerism has not. In the crystalline solid, this is not so, and we have found by X-ray structure analysis both enantiomorphic and racemate crystal structures.

In the crystal structure of dibenzoyl peroxide we find a peroxy dihedral angle of 93°. The crystals have the space group $P2_12_12_1$ and the molecules in any one crystal are all of the same sense. The same is probably true for the crystals of *p*-nitroperoxybenzoic acid, which also have the symmetry $P2_12_12_1$.

In the crystal structures of peroxypelargonic acid and *o*-nitroperoxybenzoic acid,[3] on the other hand, there are equal numbers of left and right handed molecules. In the fatty peroxy acid crystals, the molecules of the same sense are hydrogen bonded in spirals and there are an equal number of left and right handed spirals. In the *o*-nitroperoxybenzoic acid, alternate left and right handed molecules form a hy-

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drogen-bonded chain. In the latter structure all the hydrogen atoms were directly located and the dihedral angle was found to be 146°.

Thus we have observed two examples of each of the enantiomorphic and racemate type of crystal structures in the four compounds we have studied. Which crystal forms, for any particular compound, will depend on whether the assemblage of like or of unlike molecules has the greater lattice energy at the temperature of the crystallization. No other crystal structure analyses of organic peroxides have been reported. It is interesting to note, however, that hydrogen peroxide itself has an enantiomorphic crystal structure in space group $P4_12_12$,[4] while the hydrogen peroxide dihydrate structure is centrosymmetrical.[5]

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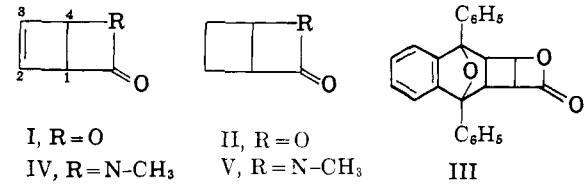
Internal Photoaddition Reactions of 2-Pyrone and N-Methyl-2-pyridone: A New Synthetic Approach to Cyclobutadiene

Sir:

Ultraviolet irradiation of a solution of 2-pyrone in ether (concentration 4–5 g./l.)[1,2] affords in almost quantitative yield an isomer to which structure I is assigned on the basis of chemical and physical data cited below. The photoproduct can be isolated in pure form by evaporation of solvent under reduced pressure (solutions of the product must be kept cold at all times) followed by evaporative distillation in a molecular still (below 0.05 mm.) at room temperature with the collecting surface maintained at −70°. It is a colorless, hygroscopic liquid which is pyrophoric in air at room temperature and which can explode on warming in air. *Anal.* Calcd. for $C_5H_4O_2$: C, 62.50; H, 4.20; mol. wt., 96. Found: C, 62.37; H, 4.20; mol. wt., 96 (mass spectral parent peak)[3]; mol. wt., 10[9] (osmometric in benzene). The n.m.r. spectrum shows four sets of peaks[4] (each due to one proton) centered at 6.73, 6.58, 5.30, and 4.40 δ, the multiplets approximating octet, quartet, quartet, and octet, respectively. The observed chemical shifts and coupling constants support structure I with the assignments of the above bands to the protons attached to C-3, C-2, C-4, and C-1, respectively (numbering as in I). The infrared spectrum (in CCl_4) of this photo 2-pyrone shows carbonyl absorption as a double peak at 5.41 and 5.50 μ (β-lactone) and a band at 6.48 μ which probably is due to C=C stretching (displaced to high wave length because of angle distortion[5]). The ultraviolet spectrum shows only end absorption (ϵ 1500 at 210 mμ). A yellow color develops with tetranitromethane, indicating the presence of an unconjugated olefinic linkage.



I, R = O II, R = O III
IV, R = N-CH$_3$ V, R = N-CH$_3$

As expected on the basis of formulation I, hydrogenation of the photoisomer (over palladium–charcoal) affords a mixture of cyclobutanecarboxylic acid[6] and a saturated β-lactone (II) (C, 61.26; H, 6.46); infrared maximum at 5.5 μ.; n.m.r. peaks (multiplets) at 4.8 (1H), 3.85 (1H), and 2.43 (4H) δ; no color with tetranitromethane. The amount of hydrogen absorbed and the ratio of products formed indicate that two molecules of hydrogen are required for the formation of the acid and one for formation of saturated lactone II.

The reaction of the photoisomer of 2-pyrone with 2,5-diphenyl-3,4-isobenzofuran[7] proceeds readily at 0° to give a 1–1 adduct, m.p. 212° dec. (C, 81.81; H, 5.10). The infrared spectrum of the adduct indicates that the β-lactone ring has been retained (band at 5.50 μ) and the n.m.r. spectrum shows (in addition to the expected aromatic proton peaks) a singlet at 3.42 δ (2H), doublets at 3.54 (1H, J = 2.6) and 4.62 δ (1H, J = 2.6), and no olefinic protons in accord with expression III for the adduct.

Photoisomerization of N-methyl-2-pyridone in ether (1.7 g./l.) under the same conditions as used for 2-pyrone follows a similar course, but at a slower rate. The product, isolated by molecular distillation at room temperature in 20% yield, is a colorless liquid (C, 65.86; H, 6.60; N, 12.92; mol. wt.,[3] 109) which is formulated as IV. The infrared spectrum shows absorption due to β-lactam carbonyl (5.74 μ) and C=C (6.49 μ) and the n.m.r. spectrum shows peaks due to two olefinic protons centered at 6.71 δ, two protons attached to saturated carbon at 4.38 and 4.18 δ, and three protons of N–CH$_3$ at 2.82 δ. The ultraviolet absorption spectrum of photo N-methyl-2-pyridone[8] shows a maximum at 237 mμ (ϵ 1500) in cyclohexane. Catalytic hydrogenation of IV produces the corresponding saturated β-lactam (V) which shows n.m.r. and infrared absorption in agreement with this structure.

Of special interest is the constitutional relationship of the internal addition products I and IV as adducts of cyclobutadiene with the stable molecules carbon dioxide and methyl isocyanate. Removal of these stable species from I and IV would seem to provide an approach to the synthesis of the elusive cyclobutadiene, either as an isolable substance or as a metastable intermediate, and this project is now under study in these laboratories. The mass spectra of I and IV provide information which is worthy of note in this regard (Table I). The photoisomer of 2-pyrone shows (in addition to the parent mass peak) m/e peaks corresponding to fragments $C_4H_4O^+$, $C_3H_3^+$, and CHO^+. These same peaks appear in the mass spectrum of furan[9] with about the same relative intensity, which suggests a pathway for fragmentation of I. Only a weak peak

(1) The irradiations were conducted using an internal water-cooled mercury arc lamp (Hanovia, Type L, 450-w.) with a Corex glass filter to eliminate wave lengths below 250 mμ. 2-Pyrone shows a maximum in the ultraviolet at 291 mμ (ϵ 7500) in ethanol. The temperature of the reaction mixture was maintained at −10 to −20° by external cooling, and the average time required for completion of the reaction was 15 hr.

(2) 2-Pyrone was prepared by pyrolysis of coumalic acid according to a procedure furnished by H. E. Zimmerman, G. L. Grunewald, and R. M. Paufler, *J. Am. Chem. Soc.*, **82**, 1514 (1960).

(3) Mass spectra were obtained using a Consolidated 21-103 C instrument.

(4) Measurements in carbon tetrachloride solution at 60 Mc., with chemical shifts given in p.p.m. downfield from tetramethylsilane as internal reference (δ).

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(8) N-Methyl-2-pyridone in ether shows absorption maxima at 233, 238, 306, 317, and 332 mμ (ϵ 5000, 3800, 4000, 3750, 1700).

(9) Catalog of Mass Spectral Data, Vol. 2, No. 508, American Petroleum Institute, Project 44.