Molecular Structure of a Stable N-Methylimidazole-Magnesium 2,4-Dinitrophenoxide Complex. A Model for Histidine and N^{τ}-Methylhistidine Coordination in MgATPases and Their Uncouplers

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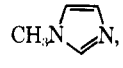
Abstract: 2,4-Dinitrophenol, the prototype of oxidative phosphorylation and muscle contraction uncouplers, forms a series of stable magnesium complexes, $(ArO)_2Mg{\cdot}L_2$. Complex stability decreases in the series L = water > *N*-methylimidazole ~ imidazole ≥ pyridine > methanol. The structure of the methylimidazole complex (**1**), $Mg(C_6H_3O_5N_2)_2(C_4H_6N_2)_2$, has been determined by single-crystal X-ray diffraction. The crystals are monoclinic, space group $P2_1/c$, a = 8.340 (4) Å, b = 11.773 (4) Å, c = 25.690 (6) Å, β = 104.65 (4)°, Z = 4, ρ_{calcd} = 1.50 g cm^{-3}, ρ_{obsd} = 1.48 g cm^{-3} (at 25 °C). The structure of the pyridine complex (**2**), $Mg(C_6H_3O_5N_2)_2(C_5H_5N)_2$, has also been determined. The crystals are triclinic, space group $P\bar{1}$, a = 8.297 (4) Å, b = 11.963 (3) Å, c = 14.345 (8) Å, α = 115.23 (4)°, β = 109.10 (4)°, γ = 81.73 (4)°, Z = 2, ρ_{calcd} = 1.49 g cm^{-3}, ρ_{obsd} = 1.47 g cm^{-3}. Intensity data were collected on a CAD4 automatic diffractometer; 4039 and 2799 independent reflections were collected using the ω-scan method (scan width of 1.0°) for **1** and **2**, respectively. The structure of **1** was solved by direct methods and refined to a final R value of 11.0% based on F for 3428 reflections by least-squares methods. The structure of **2** was solved by the Patterson superposition method and refined to a final R value of 10.5% based on F for 2132 reflections. Independent molecules with distorted octahedral magnesium hexacoordination are found for **1** and **2**. Two *o*-nitrophenoxide bidentate ligands (trans phenoxides) and two heterocyclic monodentate ligands (cis heterocycles) form the inner coordination sphere. Average distances (Å) follow: in **1**, Mg-O = 1.963 and 2.139, Mg-N = 2.115; in **2**, Mg-O = 1.953 and 2.160, Mg-N = 2.166. It is suggested that the *N*-methylimidazole-magnesium complex is a model for the possible coordination of histidine and N^{τ}-methylhistidine in certain MgATPases. The uncoupler role of 2,4-dinitrophenol may be related to the stability of its magnesium complexes.

The imidazole function of histidine residues has often been implicated in enzymatic general base and nucleophilic catalysis.[2-5] Histidine-zinc coordination has been demonstrated in several enzymes,[6] e.g., alcohol dehydrogenase,[7] carboxypeptidase,[8] and carbonic anhydrase.[9] However, to our knowledge, not much emphasis has been placed on histidine-magnesium coordination in enzymes which require this metal ion for catalysis of biophosphate reactions.[10-14] Yet it is conceivable that histidine as well as phosphate and pyrophosphate coordination with the metal could result in significant enzyme conformational changes[5,6] at or near the active site pocket, which may be essential to the enzymatic activity. This question has recently been discussed in connection with the possible role of N^{τ}-methylhistidine[15] and histidine in muscle actomyosin MgATPase.[16,17]

During our studies of magnesium coordination[18] in phosphate[19-21] and pyrophosphate[22] esters, we observed the formation of a strong complex of *N*-methylimidazole, i,

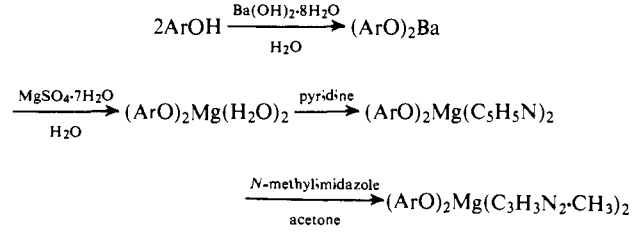


i

with a magnesium phosphodiester salt.[19] Subsequently, we confirmed the relatively high affinity of this heterocycle, and of the parent imidazole ring, toward the metal ion in a series of phenol complexes.[23] These compounds are prepared by the sequence of reactions outlined in Scheme I.

Among nitrophenols, the order of decreasing complex stability is 2,4-$(NO_2)_2$ > 2-NO_2 > 4-NO_2. For a given aroxide complex, the order of decreasing stability[46] is H_2O > *N*-methylimidazole ~ imidazole ≳ pyridine > methanol. The aquo complexes initially obtained are converted into pyridine

Scheme I



complexes upon evaporation of their pyridine solutions; however, in homogeneous acetone solution, water replaces pyridine from the corresponding complex. *N*-Methylimidazole and imidazole also replace pyridine from its complex in acetone solution. Neither alcohols larger than methanol nor phenols or thiols function as donor ligands toward magnesium aroxide salts.

The present paper describes the molecular structure of two of these compounds, di(*N*-methylimidazole)bis(2,4-dinitrophenoxide)magnesium(II) (**1**) and dipyridinebis(2,4-dinitrophenoxide)magnesium(II) (**2**), as determined by single-crystal X-ray diffraction. To our knowledge, the literature contains no structure in which an azole heterocycle functions as ligand to a magnesium oxyanion salt. There is one structure with pyridine as the ligand, namely, tetrapyridinebis(diethylphosphorothioato)magnesium(II) solved by Schwalbe, Goody, and Saenger.[24]

Experimental Section

Crystal Data. The *N*-methylimidazole compound **1**, $C_{20}H_{18}O_{10}N_8Mg$, and the pyridine compound **2**, $C_{22}H_{16}O_{10}N_6Mg$, were prepared as described.[23] The respective crystal data are given in Table I.

0002-7863/79/1501-5015$01.00/0

Table I. Crystal Data for Complexes of *N*-Methylimidazole and Pyridine with Magnesium 2,4-Dinitrophenoxide

crystal data	*N*-methylimidazole complex, **1**	pyridine complex, **2**
solvent	acetonitrile	acetonitrile
habit	rectangular prisms	rectangular prisms
system	monoclinic	triclinic
unit cell dimensions, Å, deg, Å^3	a = 8.340 (4), b = 11.773 (4), c = 25.690 (6) β = 104.65 (4) V = 2440	a = 8.297 (4), b = 11.963 (8), c = 14.345 (8), α = 115.23 (4), β = 109.10 (4), γ = 81.73 (4) V = 1217
ρ_{obsd}, g cm^{-3}	1.48[a]	1.47[a]
space group	$P2_1/c$	$P\bar{1}$
asymmetric units per unit cell	4	2
formula units per unit cell (Z)	4	2
formula unit	$Mg(C_6H_3O_5N_2)_2(C_4H_6N_2)_2$	$Mg(C_6H_3O_5N_2)_2(C_5H_5N)_2$
formula unit weight	552.7	548.8
ρ_{calcd}, g cm^{-3}	1.50	1.49
$R = \Sigma \|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	11.0%	10.5%
$R_w = [\Sigma w(\|F_o\| - \|F_c\|)/\Sigma w\|F_o\|^2]^{1/2}$ [b]	7.8%	7.4%

[a] Flotation in carbon tetrachloride-hexane. [b] $w = 1/\sigma^2(F)$.

Table IIA. Interatomic Distances (Å)[a] in Compounds **1** and **2**

bond	**1**	**2**
Mg-O(1)	1.964	1.966
Mg-O(5)	1.962	1.940
Mg-O(4)	2.152	2.186
Mg-O(2)	2.127	2.135
Mg-N(3)	2.119	2.177
Mg-N(6)	2.111	2.156
C(11)-O(1)	1.29	1.25
C(51)-O(5)	1.26	1.25
C(12)-N(12)	1.44	1.46
C(52)-N(52)	1.42	1.45
C(14)-N(14)	1.46	1.46
C(54)-N(54)	1.47	1.46
N(52)-O(2)	1.22	1.24
N(52)-O(52)	1.22	1.21
N(12)-O(4)	1.25	1.25
N(12)-O(12)	1.21	1.24
N(54)-O(541)	1.21	1.22
N(54)-O(542)	1.23	1.20
N(14)-O(141)	1.22	1.23
N(14)-O(142)	1.20	1.23

[a] The esd's for Mg-X distances are ca. 0.005 Å; for other atoms, ca. 0.01 Å.

Data Collection and Structure Determination. All the available crystals of **1** and **2** showed mosaic spread of the diffraction spots, and were not of superior quality. Crystals of ca. 0.3 × 0.3 × 0.55 (**1**) and 0.2 × 0.2 × 0.5 mm (**2**) were used to collect intensity data on an Enraf-Nonius CAD4 automatic diffractometer, employing nickel-filtered Cu Kα radiation (λ = 1.542 Å), by the ω-scan method. The scan width was 1 °, with a scan speed of 1.0° min^{-1}. Four reflections for each compound were measured periodically to monitor any crystal deterioration (three intensity control reflections) or crystal movement in the capillary tube (one orientation control reflection). If the intensity for the orientation control reflection fell ~5% from its initial value, the reflections selected for determination of cell dimensions and orientation matrix were recentered, and a new orientation matrix was determined. For the methylimidazole compound **1**, out of the 4039 independent reflections measured within $\theta \leq 65°$, 3428 reflections had their intensities greater than 3σ (σ determined from intensity counting statistics during data collection). For the pyridine compound **2**, out of 2799 measured independent reflections, 2132 reflections had their intensities greater than 3σ. Since the crystals were sealed inside a capillary tube, an empirical absorption correction[25] was applied for each crystal. The correction was determined in each case by performing an azimuth scan of a reflection occurring at $\chi \simeq 90°$. Lorentz-polarization and extinction corrections were also applied.

The structure of compound **1** was solved by direct methods.[26] One of the 64 solution sets revealed a partial structure, and the remaining atoms were located from a partial structure-phased Fourier map.

The structure of compound **2** was solved by the Patterson superposition method and iterative Fourier maps. The first superposition map performed on the most prominent nonorigin peak revealed six other peaks which had octahedral geometry around this central peak, and the positions of these peaks were at ~2 Å from this prominent peak. This arrangement was assumed to be the ligand atoms bonded to magnesium. Further superposition maps performed on these ligand peaks established the correctness of this assumption and revealed more details of the structure. The rest of the structure was determined by iterative Fourier methods.

Both structures were refined based on observed reflections by full-matrix least squares;[45] the quantity minimized was $\sum\omega(\Delta F)^2$, with weights $\omega = 1/\sigma^2(F)$. The atomic scattering factors for the atoms were obtained from International Tables.[27] Extinction corrections were applied during the anisotropic refinements. Owing to the large number of refinable parameters for each structure, the positional and thermal parameters were divided into two blocks, with those for magnesium and the immediate neighboring atoms common to both refinement groups. The positions of the hydrogen atoms were located from the difference Fourier maps. These positions were accepted as reliable because they were compatible with the calculated positions. The positional and isotropic thermal parameters of H atoms were also refined. The final difference Fourier maps revealed electron density ~1.0 e$^-$/Å^3 for either compound. This reflects a rather high background noise. However, the electron densities of H atoms in the difference Fourier maps were higher than this background; therefore, it was not difficult to locate those H atoms without ambiguity.

The refinements converged at R values of 11.0% for **1** and 10.5% for **2**, which are rather high. Evidently the unavailability of crystals of good quality is the main reason for these high values. It was found during the course of the refinements that a high percentage of the reflections were weak in **1** and **2**. Some of these reflections could have been treated as "unobserved" by setting higher threshold values for the σ level, i.e., carrying out the refinements with fewer but stronger reflections. This was regarded as an unnecessary artifact and, hence, convergence to higher R values with inclusion of all of these "weak reflections" was accepted as proper owing to the previously mentioned reasons. The final coordinates for nonhydrogen atoms in compounds **1** and **2** are given in Tables VI and VII, respectively.[28] The coordinates for hydrogen atoms in **1** and **2** are listed in Tables VIII and IX, respectively. The observed and calculated structure factors for **1** and **2** are contained in Tables X and XI, respectively.

Results

Crystals of the complexes formed by *N*-methylimidazole and pyridine with magnesium 2,4-dinitrophenoxide, **1** and **2**, contain distinct molecules in which two bidentate and two monodentate ligands coordinate to magnesium and adopt the geometry of a distorted octahedron, Figures 1 and 2. The same configuration, namely, trans phenoxides-cis heterocycles, is observed in both compounds. Table IIA gives some interatomic distances in compounds **1** and **2**; additional interatomic dis-

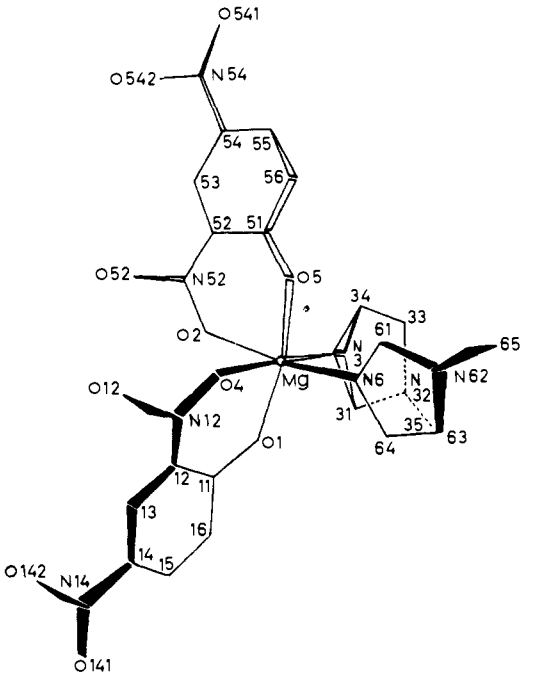


Figure 1. Computer-generated drawing of the formula unit of di(*N*-methylimidazole)bis(2,4-dinitrophenoxide)magnesium(II) (**1**).

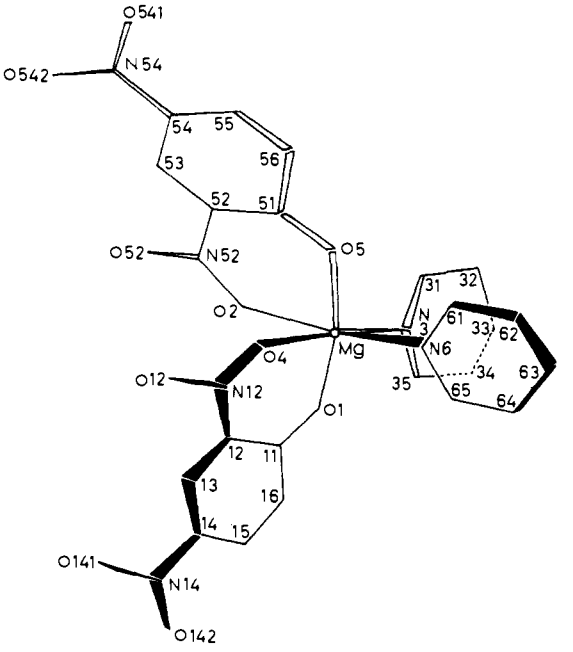


Figure 2. Computer-generated drawing of the formula unit of dipyridinebis(2,4-dinitrophenoxide)magnesium(II) (**2**).

Table IIIA. Angles (deg)[a] in Compounds **1** and **2**

angle	1	2
O(1)-Mg-O(2)	91.2	91.8
O(1)-Mg-O(4)	80.1	80.0
O(1)-Mg-O(5)	168.7	167.5
O(2)-Mg-O(4)	83.4	83.6
O(2)-Mg-O(5)	81.2	81.0
O(4)-Mg-O(5)	90.5	89.1
O(1)-Mg-N(3)	91.4	92.0
O(1)-Mg-N(6)	95.8	97.2
O(2)-Mg-N(3)	89.7	91.3
O(2)-Mg-N(6)	170.8	167.9
O(4)-Mg-N(3)	168.9	170.3
O(4)-Mg-N(6)	91.9	89.9
O(5)-Mg-N(3)	97.0	98.3
O(5)-Mg-N(6)	90.9	88.6
N(3)-Mg-N(6)	96.0	96.4
Mg-O(1)-C(11)	135.2	132.3
Mg-O(5)-C(51)	133.2	136.1
Mg-O(2)-N(52)	132.5	134.5
Mg-O(4)-N(12)	135.7	134.2
O(2)-N(52)-O(52)	119.4	120.8
O(2)-N(52)-C(52)	121.7	121.7
O(52)-N(51)-C(52)	119.0	118.4
O(4)-N(12)-O(12)	120.8	119.5
O(4)-N(12)-C(12)	120.2	121.2
O(12)-N(12)-C(12)	118.9	119.1
O(541)-N(54)-O(542)	124.4	125.1
O(541)-N(54)-C(54)	116.1	116.1
O(542)-N(54)-C(54)	119.6	118.6
O(141)-N(14)-O(142)	124.8	124.8
O(141)-N(14)-C(14)	115.6	117.4
O(142)-N(14)-C(14)	119.6	117.8

[a] The esd's of X-Mg-X angles are ca. 0.5°, and for other angles 0.8°.

Table IV. Some Intramolecular Nonbonded Distances (Å) in Compounds **1** and **2**

atoms	1	2
O(1)···O(4)	2.65	2.68
O(2)···O(5)	2.66	2.67
O(2)···O(4)	2.85	2.89
O(5)···N(6)	2.90	2.87
O(1)···O(2)	2.93	2.96
O(1)···N(3)	2.92	2.97
O(4)···O(5)	2.93	2.89
O(2)···N(3)	3.00	3.08
O(1)···N(6)	3.02	3.11
O(5)···N(3)	3.06	3.12
O(4)···N(6)	3.07	3.07
N(3)···N(6)	3.14	3.24

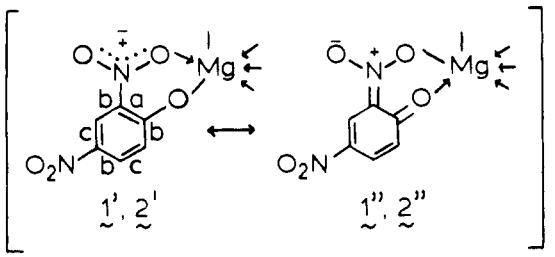
tances are recorded in Table IIB.[28] Table IIIA lists some angles in **1** and **2**, and additional angles are gathered in Table IIIB.[28] Selected nonbonded distances are listed in Table IV, and data for least-squares planes are presented in Table V.

The distortion of the octahedron is reflected in the relatively small endocyclic O-Mg-O angles. The two chelate rings are pushed away by the two heterocyclic ligands, as can be seen in the relatively small O(2)-Mg-O(4) and O(1)-Mg-O(5) angles (vs. 90 and 180°, respectively). Some of the nonbonded distances also reflect this distortion of the coordination polyhedron.

The Mg-O (phenoxide) distances are somewhat shorter (av 1.963 Å) than the Mg-O (nitro) distances (av 2.139 Å), and compare well with Mg-O (phosphate, av 2.014 Å)[20] and Mg-O (water, av 2.132 Å)[18,20] distances, respectively.[29,30] Corresponding Mg-O bonds have virtually the same length in **1** and **2**, but the Mg-N bonds are slightly shorter in the methylimidazole complex, **1** vs. **2**.

One of the two six-membered chelate rings in each of the complexes is nearly planar, but the other is somewhat distorted. Thus, planes Mg, O(1), O(4) and O(1), O(4), C(11), C(12), N(12) of compound **1** are virtually coplanar, and the magnesium lies 0.17 Å from the plane defined by the chelating atoms; however, the magnesium is 0.42 Å from the plane defined by atoms O(2), O(5), C(51), C(52), N(52). Likewise, the planes Mg, O(2), O(5) and O(2), O(5), C(51), C(52), N(52) of compound **2** are coplanar, and the magnesium is 0.01 Å from the plane of the respective chelating atoms; however, the magnesium is 0.46 Å from the other chelate plane. The structure of DNP[15] has been solved,[31] and the authors have

commented on the "o-quinonoid nature of the benzene ring" in it. This effect would be reflected in electronic structures of the type shown in formulas **1'**, **1''** and **2'**, **2''**. However, the



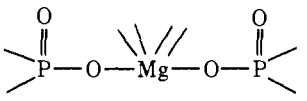
relatively high esd's in our data prevent any conclusions concerning the occurrence of this type of effect in **1** and **2**.

Discussion

The significance of this work is twofold.

(1) It shows that *N*-methylimidazole and imidazole are effective magnesium ligands and form relatively strong hexacoordinate complexes with the metal ion. This finding supports the suggestion[16,17] that the N^{τ}-methylhistidine found in skeletal muscle myosin,[32] the histidine found in place of methylhistidine in cardiac muscle myosin,[33] and the N^{τ}-methyl histidine found in the actins from all the sources so far investigated[34] have a functional role that is associated with coordination of the amino acid residues to the metal in actomyosin MgATPase. This phenomenon may be more general, and the hypothesis may also be applicable to other MgATPases and to the catalytic and/or binding processes involving other magnesium-dependent enzymes.

(2) The work also shows that DNP forms remarkably stable chelates with Mg^{2+}. This finding supports the hypothesis[16,17] that the role of this modifier of the myosin MgATPase system is associated with coordination between DNP and the metal ion at some stage of muscle contraction. Muscle contraction is basically a two-stage phenomenon in which the hydrolysis of MgATP catalyzed by the enzyme myosin releases energy which is coupled to muscle contraction by interactions between myosin and the protein actin. Actin activates the release of Pi from the myosin-ADP·Mg·Pi complex to the medium after the hydrolytic step. This "protein-product complex", myosin-ADP·Mg·Pi (but not the protein-free complex, ADP·Mg·Pi), is a high-energy compound.[16,17] In the absence of actin, the Pi-release step is rate limiting in the MgATPase pathway. The ADP and Pi moieties are strongly held together by Mg^{2+}, as has been shown[20] for the general case of the bond system ii.



ii

One of the mechanisms by which actin (the natural modifier of myosin MgATPase) can activate Pi release is by a transient coordination between the actin N^{τ}-methylhistidine residue and magnesium.[16,17] This coordination should alter the binding of the metal to Pi, ADP, and myosin, as well as the direct binding of Pi and ADP to myosin.[16,17] It is conceivable that the observed activation of Pi release and the contraction-uncoupling effect caused by DNP[35-38] result from coordination between the phenol and magnesium.[16,17] This does not preclude an additional effect of DNP on the MgATP hydrolytic step itself: $MgATP^{2-} + H_2O \rightarrow MgADP^{-} + H_2PO_4^{-}$. The evidence available at present, based on incorporation of ^{18}O into Pi from medium-$H_2{}^{18}O$, suggests that the phenol does have an effect on the mechanism of the hydrolysis, although there does not seem to be a drastic change in the mechanism type.[35-38]

The complexity of oxidative phosphorylation precludes a detailed discussion of the mechanism of its DNP uncoupling[39-44] at this time. However, to the extent that the phenol also activates Pi release in mitochondrial ATPase, a mechanistic analogy with the similar effect on myosin ATPase seems justified.

Supplementary Material Available: Tables IIB and IIIB (additional interatomic distances and angles, respectively), V (least-squares planes), VI and VII (final coordinates for nonhydrogen atoms), VIII and IX (hydrogen atomic coordinates), and X and XI (structure factors for **1** and **2**) (43 pages). Ordering information is given on any current masthead page.

References and Notes

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(15) The following abbreviations will be employed: N^{τ}-methylhistidine = 2-amino-3-(1'-methyl-4'-imidazolyl)propanoic acid; DNP = 2,4-dinitrophenol; ADP and ATP = adenosine 5'-*O*-dipoly- and tripolyphosphates, respectively; Pi = inorganic phosphate.
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(46) NOTE ADDED IN PROOF. The relatively higher stability of *N*-methylimidazole vs. imidazole complexes suggested in our preliminary communication (ref 23) has not been substantiated by further work. The present evidence suggests that both heterocycles have about the same affinity for magnesium in its complexes. In complexes of histidine and *N*$^{\pi}$-methylhistidine with magnesium phosphates in the enzyme active site pocket, the unmethylated histidine could give rise to a tighter binding than the *N*-methyl derivative for purely steric reasons.

Structure and Photochemistry of Lumiprednisone and Lumiprednisone Acetate[1a]

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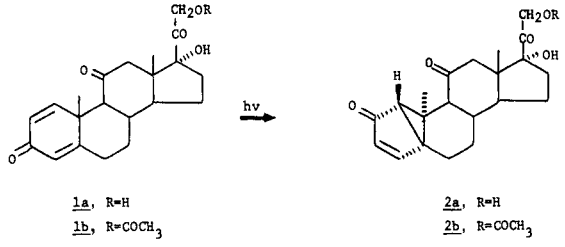
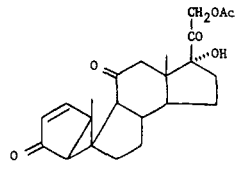
Abstract: The photolysis of prednisone acetate (**1b**) in dioxane yielded lumiprednisone acetate (**2b**) and not the previously proposed structure **3**. Further photoisomerization of **2b** in dioxane gave 21-acetoxy-2,17α-dihydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (**10b**). Irradiation of **2b** in 45% aqueous acetic acid yielded 21-acetoxy-11α,17α-dihydroxy-1β,11β-oxa-10α-pregna-2,20-dione (**14a**), which has added a molecule of water. Similar results were observed when prednisone was irradiated. Treatment of **2b** with acid afforded 17α,21-dihydroxy-1(10 → 5β)-*abeo*-pregna-1,9-diene-3,11,20-trione (**15**). The mechanism of these photoisomerization reactions is discussed. The influence of solvents and the 11-keto function on the photochemistry of the bicyclo[3.1.0]hex-3-en-2-one system is explained.

Barton and Taylor investigated the photochemistry of prednisone acetate (**1b**) more than 20 years ago, and reported its conversion into a range of novel molecules depending upon the reaction conditions.[2] Since their structure, **3**, for "lumiprednisone acetate" differed from that expected, **2b**, based on the now generally accepted mechanism for photoisomerization of cross-conjugated dienones,[3] it was decided to reinvestigate the photochemistry of the medicinally important prednisone (**1a**), its 21-acetate, **1b**, and their respective lumiproducts, **2a** and **2b**. Secondly, since photoisomerization reactions of 1,4-cyclohexadienones are extremely sensitive to changes in structure,[3] we were also interested in studying the effect of ring

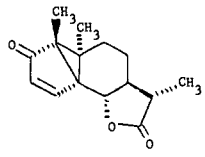


1a, R=H
1b, R=COCH$_3$

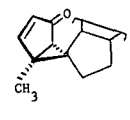
2a, R=H
2b, R=COCH$_3$



3

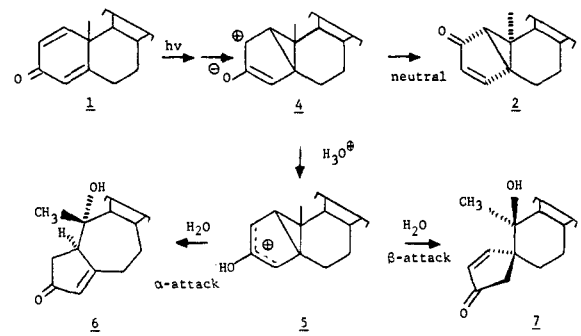


2c



3a

Scheme I. General Photoisomerization Paths of Cross-Conjugated Dienones



C substituents on these reactions. We now report a revision of the initial structural assignments and report a new photochemical reaction of lumiprednisone (**2a**) and acetate (**2b**).

It is now generally accepted that photoisomerizations of cross-conjugated cyclohexadienones take place via an n,π* excited triplet state and show a strong solvent dependence as outlined in Scheme I.[3] It was our aim to reinvestigate the photochemistry of **1** in neutral and acidic media to see if it underwent the expected photoisomerization reaction or if there was something unique about the C-11 ketone in ring C which caused the unusual results reported by Barton and Taylor.[2]

Results

A. Irradiations in Neutral Media. Irradiation of **1a** or **1b** in dry dioxane with 254-nm light afforded the "lumiprednisones" **2a** and **2b**, respectively, in 65% yield. The assignment of the structure and stereochemistry of **2a** and **2b** was by comparison of their spectral data with those of the lumiproducts, **9a** and **9b**, derived by photoisomerization of 17β-acetoxy-1-dehydrotestosterones **8a**[4] and **8b**,[5] respectively, in dioxane with 254-nm light. The structure of the lumiproduct **9a** was proven by chemical degradation[4] and by circular dichroism measurements.[6] The UV of the lumiproduct **2b**, λ_{max} 266 nm (ϵ 2500), is in close agreement with that of **9b**, 268 nm (ϵ 2950).[5] The ^{1}H NMR spectra of the A-ring protons in the lumipro-

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