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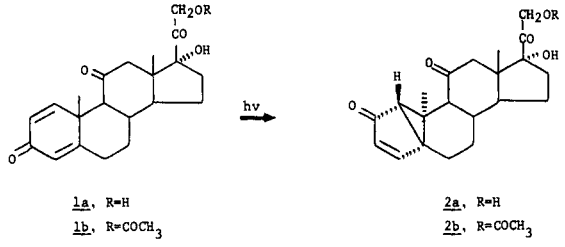
Structure and Photochemistry of Lumiprednisone and Lumiprednisone Acetate[1a]

John R. Williams,*[1b] Richard H. Moore,[1b] Rosita Li,[1b] and John F. Blount[1c]

Contribution from the Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122, and Chemical Research Division, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110. *Received December 18, 1978*

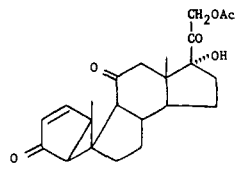
Abstract: The photolysis of prednisone acetate (**1b**) in dioxane yielded lumiprednisone acetate (**2b**) and not the previously proposed structure **3**. Further photoisomerization of **2b** in dioxane gave 21-acetoxy-2,17α-dihydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (**10b**). Irradiation 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$_3$



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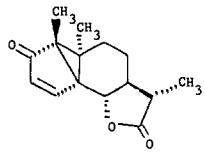
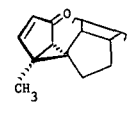
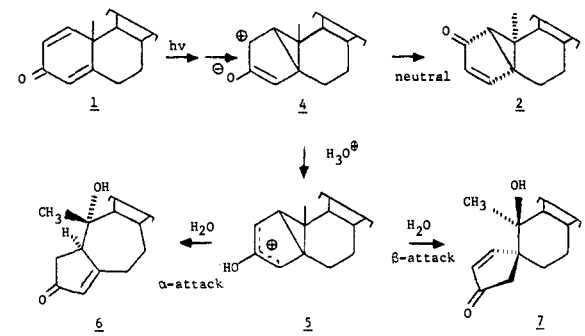


2c



3a

Scheme I. General Photoisomerization Paths of Cross-Conjugated Dienones



1 4 2 6 5 7

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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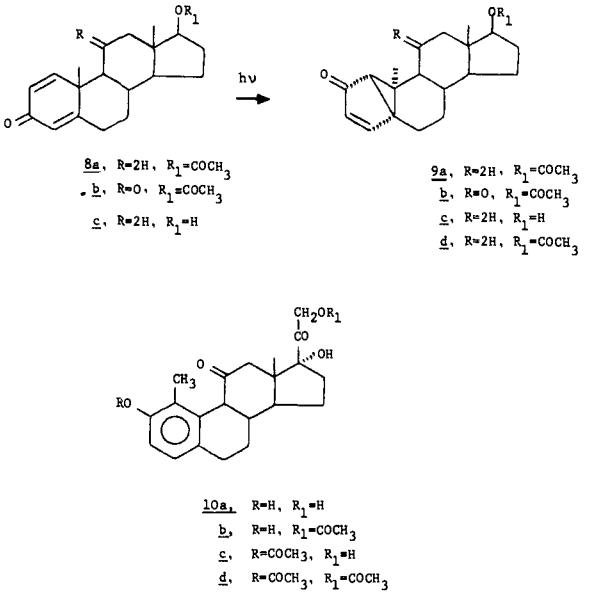
Table I. ^{13}C NMR of Lumiproducts and Related Molecules[b]

carbon	2a	2c	9c	bicyclo[3.1.0]-hex-3-en-2-one[8]	10b	cortisone acetate[a]	5,6,7,8-tetrahydro-1-naphthol	15
1	39.2	43.0	38.7	22.8	123.3	34.8†	153.2	47.6
2	211.0	206.1	204.6	193.2	153.1	33.7	112.0	203.9†
3	130.8	131.3	130.8	128.0	113.1	199.6	125.9	133.4
4	164.8	157.4	165.2	162.9	126.3	124.5	121.7	168.4
5	49.4	49.8	55.6	40.0	128.0	170.4	29.6	49.5
6	38.4	77.4	40.7		30.0	32.2*	22.8*	34.7*
7	24.0*	48.7	25.8		26.9	32.3*	22.8*	25.7
8	35.2	22.5	34.6		39.9	36.5	22.8*	37.8
9	66.0	29.6	50.6		57.2	62.5	123.7	137.0
10	27.1	40.4	24.7	35.2	132.6	38.2	138.9	142.2
11	208.2	41.3	24.6		208.7	208.7‡		208.7†
12	49.2	178.2	36.4		48.7	49.8		50.1
13	51.1	12.6	42.8		52.8	51.2		50.6
14	48.5	7.4	49.1		49.9	49.8		50.1
15	22.5*	17.1	23.1		21.6	23.2		23.8
16	33.6		29.8		34.0	35.0†		34.8*
17	87.5		80.0		87.4	88.8		89.1
18	15.7		11.3		14.8	15.4		15.7
19	14.1		14.0		14.3	17.2		16.2
20	204.6				204.8	204.3‡		204.4
21	66.0				67.6	67.3		67.5
22					169.7	168.4		170.4
23					20.2	20.4		20.4

[a] Johnson, L. F.; Jankowski, W. C. "Carbon-13 NMR Spectra", Wiley: New York, 1972. Bhacca, N. S.; Giannini, D. D.; Jankowski, W. S.; Wolff, M. E. *J. Am. Chem. Soc.* **1973**, *95*, 8421. [b] Signals marked with *,†,‡ may be reversed.

Table II. Circular Dichroism Spectra of Lumiproducts

compd	λ_{max} ($\Delta\epsilon$)	λ_{max} ($\Delta\epsilon$)	crossover λ	λ_{max} ($\Delta\epsilon$)	crossover λ	λ_{max} ($\Delta\epsilon$)
2a		343 (−4.71)	313	278 (+11.41)	253	226 (−11.11)
2b		343 (−5.21)	313	277 (+12.22)	253	225 (−11.71)
2c	355.5 (−4.21)	324 (−4.21)	309.5	275 (+11.1)	251	short wavelength negative CD
9a[4b,6]	357.5 (−3.71)	344.5 (−3.77)	309	272 (+10.3)	250	short wavelength negative CD



ducts **2a** and **2b** show a very characteristic coupling pattern similar to that described for **9a**.[4] Namely, in the NMR spectra of **2a** (**2b**) there are two doublets centered at δ 7.26 (7.20) and 5.87 (5.86) with J = 6.0 Hz, indicating the presence of an α,β-unsaturated ketone in the A ring. Double irradiation experiments indicate that the H-1 of **2a** (**2b**) is coupled to both the H-3 and H-4, through the carbonyl as was also observed in **9a**.[4] The ^{13}C NMR spectra for **2a** and a number of known lumiproducts are given in Table I. The chemical shift assignments for the carbon atoms in rings A and B of **2a** are based on those of lumisantonin (**2c**)[7] and bicyclo[3.1.0]hex-3-en-2-one,[8] and those for the C and D rings on cortisone acetate.[9] As can be seen in Table I, there is good agreement in the chemical shifts for the carbon atoms in the A and B rings of **2a** and the established lumiproduct **9c**. One would expect the radically different structure **3** to result in a significantly different ^{13}C NMR spectrum.

Proof of the stereochemistry of the lumiproducts comes from a comparison of the circular dichroism measurements on these molecules as shown in Table II. In all cases they show a negative Cotton effect of similar magnitude around 345 nm followed by a positive Cotton effect around 275 nm, again of similar magnitudes. The fine structure observed for **9a**[6] and lumisantonin (**2c**)[6] is probably due to solvent differences (dioxane vs. methanol). The CD of structures similar to **3** show no maxima or minima with an amplitude greater than 10.3.[6] Thus the CD supports structure **2** very strongly and excludes those similar to **3**.

Furthermore, it is known that the lumiproduct **2** is photoisomerized to the bicyclo[3.1.0]hexane with the configuration of the A and B rings that given in **3a**.[5] However, the CD of **3a** is opposite in sign and different in position to that of **2**, further arguing against **3** as the structure for the lumiproduct. The absence of peaks in the mass spectrum of **2a** or **2b** at $M^+ - 95$ and $M^+ - 96$ also argues against structures such as **3**.[4b] Thus the spectral data of **2a** and **2b** are consistent with those of the known lumiproducts **9a** and **9b**, and not with the spectra predicted for structure **3**.

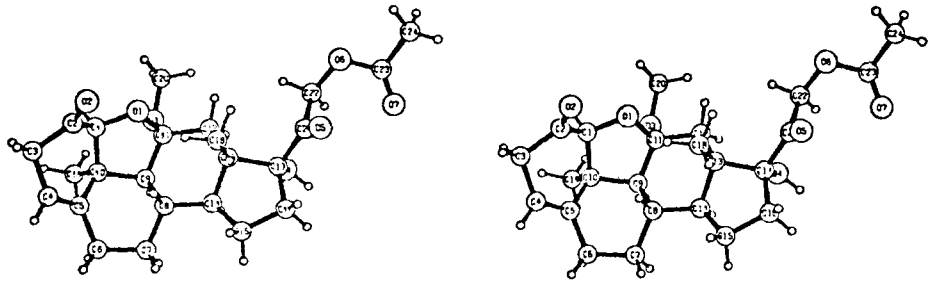


Figure 1. Conformation of **14b**.

A comparison of lumiprednisone acetate with that prepared by Barton and Taylor[10] showed them to be identical and, therefore, their structure for lumiprednisone acetate should be **2b**.

Further irradiation of **2a** and **2b** in dioxane with 254-nm light resulted in photoisomerization to the phenols **10a** and **10b**, respectively. The IR absorption at 810 cm^{-1} and the aromatic proton coupling constants of J = 8 Hz are indicative of a 1,2,3,4-tetrasubstituted aromatic ring.[2c] The chemical shift of the C-19 methyl group at δ 1.75 and 1.77 in **10a** and **10b** is upfield from that expected for a phenolic methyl group, indicating that it is in the shielding cone of the C-11 ketone and hence must be at C-1. Support for this assignment comes from the NMR spectrum of glaucanol and its derivatives.[11] When the C-11 ketone is present the C-1 methyl group absorbs at δ 1.83 ppm (glaucanol triacetate) vs. δ 2.39 for a C-11 ketal group.[11] Furthermore, a C-4 methyl group would have been expected to absorb at about δ 2.2 ppm.[11] Thus, with the methyl at C-1, the hydroxyl group must be at either C-2 or C-4 in **10**.

These two possibilities are easily distinguished by ^{13}C nuclear magnetic resonance. The ^{13}C NMR data for **10b** and the model compounds cortisone acetate (1,2-dihydroprednisone acetate) and 5,6,7,8-tetrahydro-1-naphthol are given in Table 1. The chemical shift of C-19 at δ 14.4 is upfield by 6.5 ppm from that of *m*- and *p*-cresol at δ 20.9 and 20.7, respectively.[12] In *o*-cresol, the methyl occurs at δ 16.1,[12] an upfield shift of 4.7 ppm due to the γ effect of the ortho hydroxyl group. This upfield shift of about 6.8 ppm is also observed for C-8 in 5,6,7,8-tetrahydro-1-naphthol. Thus the hydroxyl group must be at C-2 ortho to the C-1 methyl group, causing its upfield shift. Furthermore, the chemical shift of C-6 in **10b** at δ 30.0 is virtually identical with the absorption at δ 29.6 ppm for the corresponding carbon in 5,6,7,8-tetrahydro-1-naphthol, thereby supporting the position of hydroxyl group at C-2.

Further support for this assignment comes from the observation that the chemical shift difference of the methyl groups in methyl-substituted phenols in going from the phenol to the phenol acetate is very dependent upon their relationship to each other.[4b] The position of the methyl group in the phenol minus the position of the methyl in the phenol acetate, $\Delta\delta$, has the values of +0.11, −0.03, and −0.04 ppm for 1,2, 1,3, and 1,4 substitution, respectively.[4b] The $\Delta\delta$ values for **10a** and **10c**, **10b**, and **10d** were +0.14 and +0.09, respectively. Hence, the hydroxyl and methyl groups are ortho and the structures of **10a** and **10b** are assigned as having a 1-methyl-2-hydroxy A ring.

It could be argued that C-9 was epimerized during these transformations; however, it was observed in the glucanol series[11] that the C-1 methyl group is moved downfield to δ 2.00 ppm when the C-9 H is β. Since this is not the case in **10a** and **10b** they must still have the normal 9α-H configuration.

Barton and Taylor[2c] reported that the irradiation of **1b** or lumiprednisone acetate in hot dioxane affords a "para" phenol for which they proposed two possible structures, **11** and **12**. A comparison of the phenol **10b** with that prepared by Barton and Taylor[10] showed them to be identical and, therefore, their structure for the phenol should be **10b**.

B. Irradiation in Acidic Media. Irradiation of **1a** or **1b** in 50% aqueous acetic acid gave **13a** and the previously reported **13b**.[2] The absolute configuration of **13b** is epimerized at C-10 from that originally reported by Barton and Taylor[2] in light of the X-ray analysis on isophotosantonic lactone,[13] the analogous photoproduct derived from irradiation of α-santonin in acidic media. Hydrolysis of the 21-acetate in **13b** with 10% potassium bicarbonate in methanol afforded the 21-alcohol **13a**.

Since 1,4-cyclohexadienones undergo different photochemistry in neutral and acidic media, the photochemistry of the lumiproducts under these conditions was studied. It has just been shown that the lumiproducts **2a** and **2b** are further photoisomerized to the phenolic products **10a** and **10b** in neutral media. In 45% aqueous acetic acid photolysis of **2b** afforded a new steroid, **14a**, which incorporated a molecule of water. The structure of **14a** follows from its empirical formula, spectral properties, and conversion to its methoxy derivative, **14b**, by recrystallization from methanol. Spectral properties and an X-ray analysis on the methoxy derivative established its structure as **14b** (see Figure 1). Crystals of **14b** were monoclinic, space group $P2_1$, with a = 9.426 (2) Å, b = 14.027 (3) Å, c = 9.212 (2) Å, β = 97.63 (1)°, and d_{calcd} = 1.277 g cm^{-3} for Z = 2 ($C_{24}H_{32}O_7 \cdot CH_3OH$, mol wt 464.56). The intensity data were measured on a Hilger-Watts diffractometer (Ni filtered Cu Kα radiation, θ-2θ scans, pulse height discrimination). The size of the crystal used for data collection was approximately 0.3 × 0.6 × 0.7 mm. Of the 1710 independent reflections for θ < 57°, 1658 were considered to be observed ($I > 2.5\sigma(I)$). The structure was solved by a multiple solution procedure[14] and was refined by full-matrix least-squares analysis. In the final refinement anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.039 and R = 0.049 for the 1658 observed reflections. The final difference map has no peaks greater than ±0.2 e A^{-3}. Catalytic reduction of **14b** yielded dihydro-5α-**14b**.

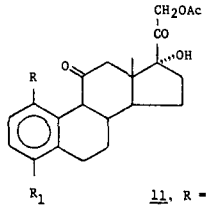
Lumiketones are known to be unstable to acid[2c] so **2b** was refluxed in 45% aqueous acetic acid for 6.5 h. Besides starting material **2b** and its hydrolysis product **2a**, a new steroid was obtained which has been assigned the structure **15** based on spectral evidence and its mode of formation from **2b** (see Discussion).

Discussion

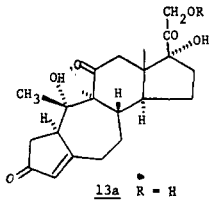
The photolysis of prednisone acetate (**1b**) in neutral media affords the expected lumiprednisone acetate (**2b**) and not the proposed structure **3**. This photoisomerization is in agreement with the now generally accepted mechanism for photochemical transformations of cross-conjugated cyclohexadienones, originally proposed by Zimmerman and Schuster.[15] Thus, the

presence of a C-11 ketone has no noticeable effect on this photoisomerization.

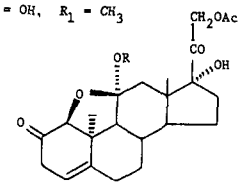
Most of the chemical transformations reported for the previously proposed structure **3** can readily be explained in terms of **2b**. Brief treatment with acetic-perchloric acid or with



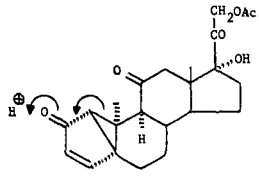
11, R = CH_3, R_1 = OH
12, R = OH, R_1 = CH_3



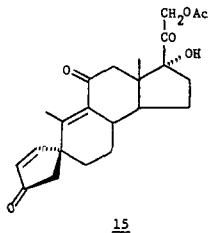
13a R = H
13b R = $COCH_3$



14a R = H
14b R = CH_3
14c R = C_2H_5

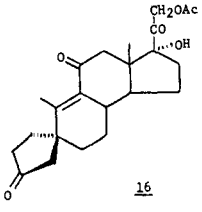


2b

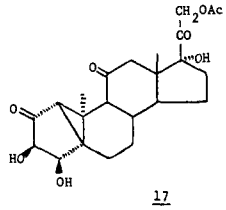


15

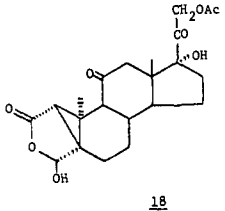
grade III alumina converts **2b** to the bisenone **15**.[2c,5] The NMR spectrum of **15** showed a pair of doublets at δ 7.56 and 6.23 with J = 5 Hz due to H-4 and H-3, respectively. The absence of any further olefinic absorption plus the presence of a methyl group at δ 1.59 are consistent with **15**. The ^{13}C NMR of **15**, given in Table I, shows the presence of two enones with the correct substitution patterns, the spiro C-5 carbon atom at δ 49.5 ppm, and is consistent with structure **15**. Catalytic reduction of **15** reduces only the cyclopentene olefin to yield **16**.[2c] Treatment of lumiprednisone acetate (**2b**) with osmium tetroxide affords the diol **17** which upon cleavage with sodium metaperiodate furnishes the neutral lactol **18**.[2c] On further oxidation with chromic acid **18** gave the pentacyclic anhydride **19** showing infrared bands at 1853 and 1773 cm^{-1}.[2c]



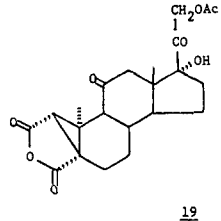
16



17



18



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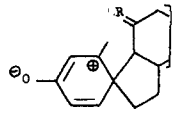
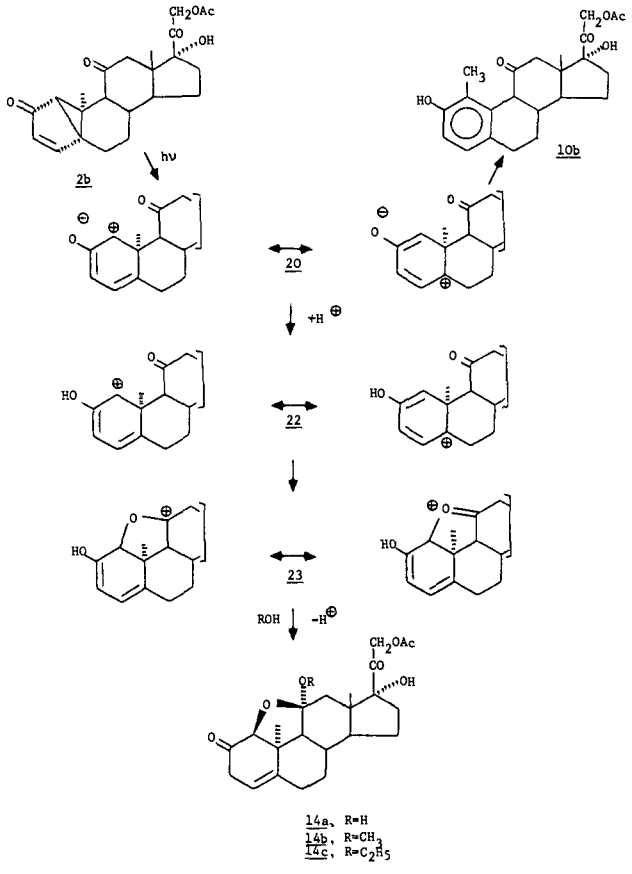


21a, R=O
b, R=2H

Wolff-Kishner reduction of the diketone **16b**, followed by chromic acid oxidation in a current of steam, was reported to give acetic acid and, in small yield, 1-methylcyclopentanecarboxylic acid.[2c] Since **16b** does contain a spirocyclopentanone system and control experiments with 4,5α-dihydrocortisone acetate gave only acetic acid, 1-methylcyclopentanecarboxylic acid could be a degradation product. Treatment of **16b** with benzylthiol in the presence of dry hydrogen chloride resulted in the addition of hydrogen chloride and formation of dithioketal. The cyclopentanone carbonyl was probably converted into the dithioketal since the thioketal gave a negative Zimmermann test and the highest carbonyl frequency, 1725 cm^{-1}, was assigned to the 21-acetate. A peak at 1710 cm^{-1} was assigned to the 11- and 20-ketones. The concomitant addition of hydrogen chloride to **16b** probably resulted in a rearrangement of the cyclopentanone ring system since treatment of the thioketal with hot pyridine did not afford an α,β-unsaturated ketone. Furthermore, removal of the dithioketal grouping with Raney nickel, followed by chromic acid oxidation, gave no trace of 1-methylcyclopentanecarboxylic acid.[2c]

Lumiprednisone (**2**) can itself be regarded as a cross-conjugated dienone in which one of the double bonds has been replaced with a cyclopropane ring. Hence, it is not surprising that it should share the same inclination for light-induced re-

Scheme II. Photoisomerization of Lumiprednisone Acetate (**2b**) in Neutral and Acidic Media



14a, R=H
14b, R=CH_3
14c, R=C_2H_5

arrangements that is exhibited by the analogous cyclohexadienones.

The formation of **10** and **14** can be explained in terms of the known photochemistry of the bicyclo[3.1.0]hex-3-en-2-one skeleton as shown in Scheme II.[3,16] The cleavage of the cyclopropyl bond which forms part of the cyclopentenone ring almost always occurs giving rise to the possible intermediate zwitterion, **20**.[3d] This explanation has received support by the observation of a blue-colored glass when **2** was irradiated at 77 K. Blue-colored intermediates[17] and cyclopropanones[18] have previously been observed when lumiproducts are irradiated at low temperatures. The intermediate can now potentially rearrange in two modes: (a) 1,2 migration of the angular methyl substituent at C-10 leading to the phenol **10** or (b) rearrangement through a spiro intermediate **21.** The choice between these two modes of rearrangement is apparently controlled exclusively by structural features and, for a given structure, is usually independent of the nature of the solvent. Thus, a 1,2 shift of the C-19 methyl group occurs when a C-11 ketone is present, whereas, when C-11 is a methylene, rearrangement through a spiro intermediate **21b** occurs leading to a 1-methyl-3-hydroxy-A-aromatic steroid.[19] The reason for this difference could be that cleavage of the 9-10 bond and migration of C-9 to the electron-deficient C-5 would involve the formation of partial positive charge on C-9. Since C-9 is adjacent to the already electron-deficient C-11 carbonyl carbon, the formation of two neighboring electron-deficient carbon atoms is avoided. Similar results have been observed for the acid-catalyzed dienone-phenol rearrangement of steroidal 1,4-diene-3,11-diones.[20]

When **2** is irradiated in protic solvents a new reaction occurs leading to the formation of **14a** which has added a molecule of water. Studies on analogous systems have indicated that the zwitterion **20** may be protonated on oxygen leading to the carbonium ion **22**.[16] Attack by carbonyl oxygen on C-1 would then lead to a 1-oxacyclopent-1-enyl cation **23,** which would be trapped by the solvent. To our knowledge this is the first time that a 1-oxacyclopent-1-enyl cation has been generated photochemically, although they are known.[21] Loss of a proton then affords the new steroid skeleton **14a**. Intermolecular nucleophilic trapping of possible zwitterionic intermediates generated by irradiation of cross-conjugated dienones is known.[22,3g]

In summary, this new reaction pathway shows that the 11-keto group does not affect the usual photoisomerization reactions of steroidal cross-conjugated cyclohexadienones, but it does influence the photochemistry of their lumi intermediates.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. IR spectra were taken in KBr with a Perkin-Elmer 225 spectrophotometer. UV absorption spectra were measured in methanol on a Cary 14 spectrophotometer. NMR spectra were recorded at 100 MHz on a Varian XL-100 spectrometer fitted with a Nicolet NTCFT 1180 pulse system and at 90 MHz on a Perkin-Elmer R32 spectrometer. Chemical shifts are reported in δ (ppm) from the internal standard Me_4Si in chloroform-*d* with a minimum of dimethyl-d_6 sulfoxide added for solubility, unless otherwise stated. Optical rotations were measured on a Rudolph Model 63 polarimeter, equipped with a photoelectric indicator, using a 1-dm cell with methanol as the solvent. Circular dichroisms were measured on a Jasco J-41A spectropolarimeter. TLC was carried out on silica gel GF plates using 10% methanol in chloroform as the eluent. Analytical LC was carried out on a Waters Associates 1/4 in. × 25 cm μC_{18} column, eluting with 50% aqueous methanol. Preparative LC was carried out using a 1/2 in. × 50 cm column packed with Waters Associates 37-75 μ Porasil A, eluting with 1-10% methanol in chloroform. Prednisone (Upjohn Co.) had mp 232-234 °C (lit.[23] 233-235 °C); prednisone acetate (Upjohn Co.) had mp 234-235 °C (lit.[23] 226-232 °C). 1,4-Dioxane was purified by refluxing over sodium for 24 h followed by distillation under argon.

Lumiprednisone (2a). Prednisone (**1a**, 1.000 g, 2.790 mmol) was dissolved in 60 mL of purified 1,4-dioxane. The solution was flushed with argon, stirred, and irradiated with a low-pressure mercury lamp (2.5 W Hanau) for 3.0 h. The reaction was monitored by analytical LC. The solvent was evaporated in vacuo to give a solid which upon recrystallization from ethyl acetate gave lumiprednisone (**2a,** 0.473 g, 47%): mp 225-226 °C dec; $[\alpha]_D$ −88° (*c* 1.22); UV (ethanol) λ_{max} 265 nm (ϵ 1870) and 215 (4480); IR 1710 (C=O), 1680 (C=O), 1570 (C=C), 1442 (-CH_3), and 1049 cm^{-1} (cyclopropyl); NMR δ 7.26 (d, J = 6 Hz, 1, H-4), 5.87 (d, J = 6 Hz, 1, H-3), 5.40 (s, 1, exchanges with D_2O, -OH), 4.9-4.1 (complex, 3, exchanges with D_2O giving an AB quartet at 4.22, 2, J = 19.5 Hz, $\Delta\nu$ = 43 Hz, H-21), 1.79 (s, 1, H-1), 1.16 (s, 3, H-19), 0.53 (s, 3, H-18), 3.1-1.2 (complex); MS *m/e* (rel intensity) 358 (2, M^+), 340 (2), 328 (2), 298 (3), 255 (2), 187 (4), 186 (2), . . . , 97 (3), 96 (2), 94 (2), 93 (3), 92 (2), 91 (9).

Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.46; H, 7.27. The filtrate was concentrated and separated by preparative LC to give **2a** (0.144 g, total yield 62%) and **10a** (0.13 g, 11%).

Lumiprednisone Acetate (2b). Prednisone acetate (**1b,** 800 mg, 2.0 mmol) was photolyzed in 45 mL of dioxane as described above for **1a**. Recrystallization of the crude photoproduct from ethyl acetate gave 520 mg (65%) of lumiprednisone acetate (**2b**) as colorless flakes: mp 231-234 °C dec; $[\alpha]_D$ −70° (*c* 2.30); UV (ethanol) λ_{max} 266 nm (ϵ 2500); IR 1740 (C=O, acetate), 1725 (C=O), 1700 (C=O), 1570 (C=C), and 1047 cm^{-1} (cyclopropyl); NMR δ 7.19 (d, J = 6 Hz, 1, H-4), 5.85 (d, J = 6 Hz, 1, H-3), 4.90 (AB quartet, J = 17.5 Hz, $\Delta\delta$ = 43 Hz, 2, H-21), 3.62 (s, 1, OH), 2.16 (s, 3, acetate), 1.85 (s, 1, H-1), 1.22 (s, 3, H-19), and 0.64 (s, 3, H-18); MS *m/e* (rel intensity) 401 (6), 400 (19, M^+), 370 (11), 358 (6), 340 (5), 328 (3), 299 (4), 281 (4), 253 (4), . . . , 97 (6), 96 (2), 95 (8), 94 (5), 93 (11), 92 (5), 91 (24) . . . .

Anal. Calcd for $C_{23}H_{28}O_6$: C, 69.06; H, 7.05. Found: C, 69.02; H, 6.80.

Lumiprednisone acetate:[2c] plates (from methanol); mp 224-226 °C; $[\alpha]_D$ −84° (*c* 0.80); IR 1735, 1708, 1690, and 1575 cm^{-1}; UV λ_{max} 218 and 265 nm (ϵ 5900 and 2300, respectively).

A mixture melting point, identical chromatographic behavior, infrared spectra, and mass spectra of the materials in question indicated that lumiprednisone acetate obtained by Barton and Taylor[2c,10] was identical with **2b**.

Lumisantonin (2c). Santonin (1.000 g, 4.061 mmol) was dissolved in 120 mL of purified dioxane and irradiated under nitrogen for 210 min using a 2.5-W low-pressure mercury lamp. Dioxane was removed in vacuo, and the solids were recrystallized from ethanol to give 801 mg of lumisantonin (**2c,** 3.245 mmol, 80%): mp 153-154 °C (lit.[24] mp 153-155 °C); IR (KBr) 1765, 1701, 1660 (shoulder), 1574, 1165, 990, 742 cm^{-1} (lit.[24] 1765, 1703, 1660 cm^{-1}); NMR ($CDCl_3$) δ 7.65, (d, J = 6 Hz, 1, H-4), 6.03 (d, J = 6 Hz, 1, H-3), 3.98 (d, J = 11 Hz, 1, H-6), 1.26 (d, J = 8 Hz, 3, H-13), 1.24 (s, 3, H-15), 1.15 (s, 3, H-14), 2.6-1.0 (complex, 6).

Lumidehydrotestosterone (9c). 1-Dehydrotestosterone (**8c,** 1.50 g, 5.24 mmol) was dissolved in 120 mL of purified dioxane and irradiated under nitrogen for 240 min using a 2.5-W low-pressure mercury lamp. Dioxane was removed in vacuo. Chromatography over silica gel, eluting with 30% ethyl acetate in hexane (v/v), gave 894 mg of **8c** (3.12 mmol, 59.6% recovery), mp 163-165 °C (lit.[25] 164-166 °C), and 549 mg of lumidehydrotestosterone (**9c,** 1.92 mmol, 36.6%) as an oil.

Lumidehydrotestosterone Acetate (9d). Acetylation of 549 mg of **9c** (1.917 mmol) with acetic anhydride in pyridine gave, after recrystallization from ethanol, 438 mg of lumidehydrotestosterone acetate (**9d,** 1.334 mmol, 70%): mp 159-160 °C (lit.[4a] 161-162 °C); IR 1727, 1686, 1665 (shoulder), 1565, 1239, and 1041 cm^{-1} (lit.[4a] 1739, 1693, 1669, 1640, and 1255 cm^{-1}); NMR δ 7.28 (d, 1, J = 5.5 Hz, H-4), 5.89 (d, 1, J = 5.5 Hz, H-3), 4.65 (t, 1, J = 8.5 Hz, H-17), 1.03 (s, 3, H-21), 1.90 (s, H-1), 1.21 (s, 3, H-18), 0.82 (s, 3, H-19), and 2.2-0.9 (complex) (lit.[4a] 7.20, 5.82, 4.59, 2.03, ca. 1.9, 1.22, 0.81).

2,17α,21-Trihydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (10a). Prednisone (**1a,** 600 mg, 1.67 mmol) in 150 mL of dry dioxane was irradiated under a nitrogen atmosphere with a 450-W high-pressure mercury lamp using a uranium glass filter for 14 h. Evaporation of the solvent in vacuo followed by crystallization from ethanol gave 334 mg (0.932 mmol, 56%) of 2,17α,21-trihydroxy-1-

methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (**10a**) as colorless needles: mp 257–258 °C dec; $[\alpha]_D$ +224° (*c* 3.02); UV (ethanol) λ_{max} 284 nm (ϵ 2040) and 214 (7950), becoming 300 (4900) and 243 (12 100) upon addition of base; IR 1710 (C=O), 1589 (aromatic), 1483 (aromatic), and 808 cm^{-1} (1,2,3,4-tetrasubstituted aromatic); NMR δ 8.45 (s, 1, exchanges with D_2O, ArOH), 6.69 (q, 2, J = 8.3 Hz, $\Delta\nu_{AB}$ = 6.6 Hz, H-3,4), 5.47 (s, 1, exchanges with D_2O, OH), 4.7–3.9 (m, 3, upon addition of D_2O gives an AB quartet centered at 4.40, 2, J = 18.5 Hz, $\Delta\nu$ = 46 Hz, H-21), 3.24 (d, 1, J = 10 Hz, H-9), 2.8–1.4 (m), 1.75 (s, 3, H-19), 0.59 (s, 3, H-18).

Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.46; H, 7.57.

21-Acetoxy-2,17α-dihydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (10b). Prednisone acetate (**1b**, 600 mg, 1.50 mmol) in 150 mL of dry dioxane was photolyzed as above. Evaporation of the solvent in vacuo, followed by chromatography on a silica gel column, eluting with 5% methanol in chloroform (v/v), gave 375 mg (0.936 mmol, 63%) of 21-acetoxy-2,17α-dihydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (**10b**) as colorless needles: mp 267–270 °C dec (lit.[2c] 258–265 °C); $[\alpha]_D$ +209° (*c* 3.04); UV λ_{max} 284 nm (ϵ 2100) and 214 (7950), becoming 303 (3190) and 242 (8860) upon addition of base; IR 1750, 1720, 1700, 1625, 1600, and 810 cm^{-1} (1,2,3,4-tetrasubstituted aromatic); NMR δ 8.37 (s, 1, OH), 6.67 (AB quartet, J = 7.5 Hz, $\Delta\nu$ = 7.3 Hz, 2, H-3,4), 5.48 (s, 1, OH), 4.92 (AB quartet, J = 13 Hz, $\Delta\nu$ = 22 Hz, 2, H-21), 3.66 (d, J = 10 Hz, 1, H-9), 3.27 (d, J = 10 Hz, 1, H-8), 3.12 (s, 1, OH), 2.14 (s, 3, acetate), 1.78 (s, 3, H-19), 0.62 (s, 3, H-18), and 2.9–1.3 (m).

Anal. Calcd for $C_{23}H_{28}O_6$: C, 69.05; H, 7.05. Found: C, 69.06; H, 6.98. Use of a Pyrex instead of glass filter in the above reactions resulted in lower yields of **10a** and **10b**.

Photoisomerization of 2a to 10a and 2b to 10b. The lumiprednisones **2a** and **2b** (150 mg) in 100 mL of dry dioxane were irradiated with a 2.5-W Hanau low-pressure mercury lamp for 1 h. The solvent was removed in vacuo and crystallization of the residue from ethyl acetate gave **10a** and **10b** in 77 and 81% yields, respectively. **10a** and **10b** were identical by melting point, mixture melting point, and comparison of infrared spectra with samples of **10a** and **10b** prepared previously.

2,21-Diacetoxy-17α-hydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (10d). Acetylation of **10b** with acetic anhydride–pyridine yielded 2,21-diacetoxy-17α-hydroxy-1-methyl-19-norpregna-1,3,5(10)-triene-11,20-dione (**10d**) as colorless needles: mp 240–243 °C; $[\alpha]_D$ +310° (*c* 3.49); UV (ethanol) λ_{max} 267 nm (ϵ 550) and 276 (544); IR 1750, 1730, 1700, and 1625 cm^{-1}; NMR δ 6.83 (AB quartet, J = 8.5 Hz, $\Delta\nu$ = 13 Hz, 2, H-3,4), 6.00 (s, 1, OH), 4.98 (AB quartet, J = 17 Hz, $\Delta\nu$ = 22.5 Hz, 2, H-21), 3.71 (d, J = 10 Hz, 1, H-9), 3.33 (d, J = 10 Hz, 1, H-8), 2.27 (s, 3, 2-acetate), 2.10 (s, 3, 21-acetate), 1.72 (s, 3, H-19), 0.60 (s, 3, H-18), 2.9–1.2 (m).

Anal. Calcd for $C_{25}H_{30}O_7$: C, 67.85; H, 6.80. Found: C, 67.77; H, 6.88.

9aα,17α,21-Trihydroxy-9aβ-methyl-19-nor-*A*-nor-*B*-homo-10α-pregn-3-ene-2,11,20-trione (13a). Prednisone (**1a**, 3.177 g, 8.863 mmol) was dissolved in 1250 mL of aqueous acetic acid (50% v/v). This solution was stirred and irradiated for 2 h with a 450-W high-pressure Hanovia lamp in a Pyrex well. The photolysis was monitored by analytical LC. The solvent was evaporated under reduced pressure to give a solid. Recrystallization from methanol–ethyl acetate gave 1.670 g (50% yield) of the trione **13a**; mp 234–235 °C dec; $[\alpha]_D$ +152° (*c* 3.82); UV (ethanol) λ_{max} 233 nm (ϵ 31 100); IR 1700 (C=O), 1679 (C=O), 1602 cm^{-1} (C=C); NMR δ 5.93 (s, 1, H-3), 5.47 (s, 1, exchanges with D_2O, -OH), 4.89 (s, 1, exchanges with D_2O, OH), 4.75–4.0 (m, 3, part exchanges with D_2O giving an AB quartet at 4.40, 2, J = 19.5 Hz, $\Delta\nu$ = 40 Hz, H-21), 1.09 (s, 3, H-19), 0.58 (s, 3, H-18), 3.4–1.1 (m).

Anal. Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 66.91; H, 7.54.

The filtrate was concentrated and separated by preparative LC to give 0.849 g (27%) of prednisone (**1a**) and 0.367 g (total yield 83.3%) of dione **13a**.

21-Acetoxy-9aα,17α-dihydroxy-9aβ-methyl-19-nor-*A*-nor-*B*-homo-10α-pregn-3-ene-2,11,20-trione (13b). Prednisone acetate (**1b**, 310 mg, 0.77 mmol) in 80 mL of aqueous acetic acid (45% v/v) was stirred with nitrogen and irradiated for 3 h with a 450-W high-pressure mercury lamp using a Corex filter. Evaporation of the solvent in vacuo followed by recrystallization from methanol gave 228 mg (74% yield) of 5,7-fused hydroxy ketone **13b** as colorless prisms: mp 247–248 °C (lit.[2c] 240–243 °C); $[\alpha]_D$ +154° (*c* 1.79); UV (ethanol) λ_{max} 231 nm (ϵ 18 000); IR 1742, 1721, 1675, and 1601 cm^{-1}; NMR δ 5.98 (s, 1, H-3), 5.60 (broad, 1, OH), 4.90 (AB quartet, J = 15.5 Hz, $\Delta\nu$ = 20 Hz, 2, H-21), 4.88 (s, 1, OH), 2.14 (s, 3, acetate), 1.10 (s, 3, H-19), and 0.58 (s, 3, H-18).

Hydrolysis of 13b. To a solution of 200 mg (0.48 mmol) of **13b** in 25 mL of methanol, 5 mL of potassium bicarbonate solution (10%) was added dropwise. The reaction mixture was stirred at room temperature for 10 min, concentrated in vacuo, and extracted with chloroform. The chloroform extract was dried and concentrated in vacuo to give 136 mg (68% yield) of 21-hydroxy compound **13a**. Crystallization from methanol gave **13a** as colorless prisms, mp 226–228 °C, mmp 227–229 °C. The infrared spectrum of compound **13a** was identical with that prepared by direct irradiation of **1a**.

21-Acetoxy-11α,17α-dihydroxy-1β,11β-oxo-10α-pregn-4-ene-2,20-dione (14a). A solution of 2.0 g (5.0 mmol) of lumiprednisone acetate (**2b**) in 475 mL of 45% aqueous acetic acid (v/v) was irradiated with a 450-W Hanovia high-pressure mercury lamp through a Pyrex filter for 35 min. Concentration of the solvent in vacuo and recrystallization from ethyl acetate afforded 1.20 g (2.37 mmol, 47%) of a colorless, crystalline product (**14a**) containing a molecule of ethyl acetate: mp 184–187 °C dec, 119–122 °C phase change; $[\alpha]_D$ −77° (*c* 2.24); UV (methanol) λ_{max} 288 nm (ϵ 209); IR 3450, 1740, 1725, 1630, 1050, 1010 cm^{-1}; NMR δ 5.37 (d, 1, J = 7 Hz, H-4), 5.35 (s, 1, exchanges with D_2O, OH), 4.97 (AB quartet, 2, J = 19 Hz, $\Delta\nu$ = 24 Hz, H-21), 5.05 (s, 1, exchanges with D_2O, OH), 4.08 (q, 2, J = 7 Hz, ethyl acetate methylene), 2.09 (s, 3, 21-acetate), 1.98 (s, 3, acetate), 1.60 (s, 3, H-19), 1.22 (t, 3, J = 7 Hz, ethyl acetate methyl), 0.63 (s, 3, H-18).

Anal. Calcd for $C_{27}H_{38}O_9$: C, 64.01; H, 7.56. Found: C, 64.40; H, 7.49.

21-Acetoxy-11α-methoxy-1β,11β-oxo-10α-pregn-4-ene-2,20-dione (14b). When the dihydroxy steroid **14a** is recrystallized from methanol or lumiprednisone acetate (**2b**) is photolyzed as above but recrystallized from methanol, the 11α-methoxy steroid **14b** is formed in 60% yield: mp 128–130 °C dec, 108–112 °C phase change; $[\alpha]_D$ −95° (*c* 1.46); UV (methanol) λ_{max} 289 nm (ϵ 197); IR 3450, 1740, 1725, 1630, 1075, 1050, and 1005 cm^{-1}; NMR δ 5.44 (d, 1, J = 7 Hz, H-4), 4.94 (AB quartet, 2, J = 18 Hz, $\Delta\nu$ = 48 Hz, H-21), 4.23 (s, 1, H-1), 3.30 (s, 3, OCH_3), 2.14 (s, 3, acetate), 1.59 (s, 3, H-19), and 0.77 (s, 3, H-18).

Anal. Calcd for $C_{25}H_{36}O_8$: C, 64.63; H, 7.81. Found: C, 64.23; H, 7.84.

21-Acetoxy-17α-hydroxy-11α-ethoxy-1β,11β-oxo-10α-pregna-2,20-dione (14c). A solution of 2.00 g (5.00 mmol) of prednisone acetate (**1b**) in 475 mL of absolute ethanol was irradiated under nitrogen with a 2.5-W low-pressure mercury lamp for 10 h. The solvent was removed in vacuo and the product was chromatographed on a silica gel column, eluting with 1% methanol in chloroform (v/v), to give 1.365 g of **14c** (3.057 mmol, 61%). Recrystallization from ethanol gave 570 mg (1.277 mmol, 26%) of **14c**: mp 148–152 °C dec, 99–102 °C phase change; $[\alpha]_D$ −60° (*c* 0.50); UV (methanol) λ_{max} 283 nm (ϵ 356); IR 3470 (OH), 1722 (C=O), 1371, 1260, 1229, and 1043 cm^{-1}; NMR δ 5.38 (d, 1, J = 7 Hz, H-4), 4.93 (AB quartet, 2, J = 17.5 Hz, $\Delta\nu$ = 45 Hz, H-21), 4.22 (s, 1, H-1), 3.64 (q, 2, J = 6 Hz, $-OCH_2-$), 3.34 (br, 1, OH), 2.20 (s, 2, H-3), 2.13 (s, 3, acetate), 1.61 (s, 3, H-19), 1.18 (t, 3, J = 6 Hz, $-OCH_2CH_3$), 0.72 (s, 3, H-18).

Anal. Calcd for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.11; H, 7.85.

17α,21-Dihydroxy-1(10 → 5β)-*abeo*-pregna-3,9-diene-2,11,20-trione (15). A solution of 134 mg (0.36 mmol) of lumiprednisone acetate (**2b**) in 25 mL of aqueous acetic acid (45%) was refluxed under nitrogen for 6.5 h. The solution was evaporated and the residue was chromatographed on a preparative LC using acetone–chloroform (2:98) as eluent. This gave 59 mg (34%) of starting material, **2b**, 29 mg (23%) of lumiprednisone (**2a**), and 15 mg (0.042 mmol, 12%) of the spirohydroxy derivative **15**. Crystallization from ethyl acetate gave colorless crystals: mp 200–202 °C; UV (ethanol) λ_{max} 235 nm (ϵ 11 800); IR 1725, 1720, 1675, 1598, and 1580 cm^{-1}; NMR δ 7.56 (d, J = 5 Hz, 1, H-4), 6.23 (d, J = 5 Hz, H-3), 5.39 (s, 1, OH), 6.8–6.0 (complex, 3, exchanges with D_2O giving an AB quartet, J = 22 Hz, $\Delta\nu$ = 39 Hz, 2, H-21), 1.59 (broad singlet, 3, H-19), and 0.66 (s, 3, H-18).

Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.37; H, 7.31. Found: C, 70.23; H, 7.32.

21-Acetoxy-17α-hydroxy-11α-methoxy-1β,11β-oxo-5α,10α-pregna-2,20-dione. To a solution of 300 mg of **14b** in 30 mL of ethanol

was added 60 mg of 10% Pd on charcoal. The mixture was hydrogenated at room temperature and pressure overnight and absorbed 15.8 mL (approximately 1 equiv) of hydrogen. The catalyst was filtered off and the filtrate evaporated to dryness. Attempts to recrystallize it were unsuccessful: mp 113–115 °C, 93–96 °C phase change; $[\alpha]_D$ +53° (*c* 3.59); UV (methanol) λ_{max} 288 nm (ϵ 209); IR 3450, 1750, 1720, and 1630 cm^{-1}; NMR δ 4.93 (AB quartet, 2, J = 18 Hz, $\Delta\nu$ = 50 Hz, H-21), 4.06 (s, 1, H-1), 3.25 (s, 3, OCH_3), 2.14 (s, 3, acetate), 1.61 (s, 3, H-19), and 0.81 (s, 3, H-18).

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Physical, Chemical, and Enzymological Characterization of Enolpyruvate[1]

Donald J. Kuo, Edward L. O'Connell, and Irwin A. Rose*

Contribution from The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received March 26, 1979*

Abstract: Acid and alkaline phosphatase reacts with phosphoenolpyruvate (PEP) to generate enolpyruvate, but neither ketopyruvate nor the geminate diol of pyruvate. The adsorption spectrum for the phosphatase product was derived by kinetic correlation of the changing spectra. Its λ_{max} (225 nm) and molar absorptivity, ϵ_{225} 9600 M^{-1} cm^{-1}, are appropriate for 2-hydroxyacrylic acid. Mass spectral analysis shows that the phosphatase product that accumulates transiently requires the addition of a proton to C-3 to give pyruvate. The protonation is slowed ca. sixfold in D_2O compared to H_2O. Enolpyruvate has sufficient stability ($t_{1/2}$ = 3.6 min at 20 °C in D_2O, pD 6.4) to be examined as a possible intermediate in enzymatic catalysis. It was predictably shown to lead to strong inhibition of lactate dehydrogenase in the presence of DPN^+. Catalysis by pyruvate kinase of the ketonization of enolpyruvate, generated with phosphatase in situ, was shown to occur with $k_{cat.}$ = 50 min^{-1} and to require both K^+ and Mg^{2+}. The apparent K_M is $\sim 10^{-8}$ M. The rate of ketonization is about 11% of the rate of the overall reaction: ADP + PEP → ATP + pyruvate.

Introduction

The enolic form of pyruvate may be considered a possible transient intermediate in many enzyme-catalyzed reactions specific for phosphoenolpyruvate (addition at C-3 or replacement of the $-PO_3$ group) and pyruvate (substitution at C-3).[2] We have recently reported[3] that the product of phosphatase action on phosphoenolpyruvate (PEP), presumed to be enolpyruvate, is converted stereospecifically to pyruvate by muscle pyruvate kinase. In the present paper we provide evidence of the physical and chemical nature of the phosphatase product that characterizes it as enolpyruvate. In addition, further studies with pyruvate kinase are reported indicating the kinetic properties and cofactor requirements for the ketonization.

Experimental Section

Materials. Phosphoenolpyruvate (tricyclohexylamine salt) and L-lactate dehydrogenase (LDH, EC 1.1.1.27) from beef heart were purchased from Sigma Chemical Co. and D-lactate dehydrogenase (EC 1.1.1.28) of *Lactobacillus leichmannii* was from Bochringer. When PEP was used for spectral studies, it was passed through Dowex AG 50W-X4 (H^+ form, Bio-Rad) and Chelex-100 to remove cyclohexylammonium cation and contaminating metal ions. Acid phosphatase from potatoes (type IV of Sigma or grade I of Boehringer) and alkaline phosphatase from calf intestine (type VII of Sigma) or *Escherichia coli* (type IIIS of Sigma) could be used interchangeably as a source of enolpyruvate at pD 6. Pyruvate kinase (EC 2.7.1.40) from rabbit muscle was obtained from Boehringer-Mannheim Biochemicals. The ammonium sulfate suspension of each enzyme was centrifuged at 15000*g* for 10 min, taken up in 99.8% D_2O (Aldrich

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