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The Synthesis, Structure and Properties of a 1, 2-Dihydropyrimidin-2-one formed by UV-Irradiation of 5-*t*-Butyl-1-Methyluracil

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Abstract: Photoirradiation of 5-*t*-butyl-1-methyluracil at 254 nm results in the production of a 1,2-dihydro-1-methyl-pyrimidin-2-one in high yield. The structure was confirmed by X-ray analysis. When subjected to alkaline conditions, a bathochromic shift in the UV spectrum accompanied by an increase in molar absorptivity, typical of 1,2-dihydro-1-substituted pyrimidin-2-one, was observed. For the first time we can postulate that the compound responsible for this UV spectrum is the intermediate formed following attack of hydroxide ion at C-6, followed by breakage of the N-1→ C-6 bond to yield an α, β-unsaturated aldehyde. Copyright © 1996 Elsevier Science Ltd

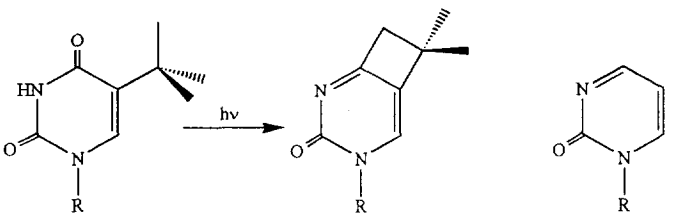
UV-irradiation of DNA is known to lead to mutagenic events and hence because of concerns about radiation damage, pyrimidine bases and their nucleosides have long been a subject of interest to determine the products formed by such radiation. Thus the phenomena of photohydration of uracil and dimer formation from thymine derivatives have been known for a long time and are well understood.

In the past 20 years, many 5-substituted pyrimidine nucleosides have been synthesised following the pioneering work of Prusoff[1] who made 5-iodo-2′-deoxyuridine which was shown to possess antiviral properties. Many other such analogues have also been shown to interfere with viral nucleic acid replication (particularly herpes virus) and many of these compounds such as 5-ethyl-[2], 5-vinyl-[3] and *E*-5-(2-bromovinyl)-2′-deoxyuridine[4] have substituents containing more than one carbon atom. When such analogues as 5-ethyl-, 5-isopropyl-, 5-hexyl-2′-deoxyuridine are subjected to UV-irradiation, *cis*-2, 4-diazabicyclo[4.2.0]octan-3, 5-diones are formed.[5] These compounds, which are also the cycloaddition products of photoirradiation of uracil derivatives and olefins,[6] hence react further to give 2′-deoxyuridine which then photohydrates. The presence of another unidentified photoproduct with λ_{max} 307 nm, shifting to 348 nm upon addition of alkali, was also reported.[7]

Recently we have described a novel and unexpected photochemical reaction of 5-t-butyl-2′-deoxyuridine **3** which gives a high yield of the 2′-deoxynucleoside of a cyclobutan-1, 2-dihydropyrimidin-2-one **5**,[8] the structure of which was elucidated by spectroscopic techniques. This nucleoside has been shown to be an inhibitor of cytidine deaminase.[9]

We here describe the effect of UV-irradiation upon N-1-methyl-5-*t*-butyluracil **2** to give the corresponding N-1-methyl-substituted cyclobutanpyrimidin-2-one **4** (Scheme 1). This photoproduct could be

crystallised and its structure determined by X-ray analysis. The photoproduct had very similar spectral properties to the deoxynucleoside previously isolated and it confirms our previous assignment of the structure. We are now able to follow the reaction of the N-1-methyl photoproduct with alkali and have shown that the spectral changes seen (307→ 348 nm) are consistent with a previously proposed reaction pathway for the reaction of hydroxide ion with 1, 2-dihydropyrimin-2-ones, involving initial attack at C-6 and opening of the pyrimidinone ring.[10] The final products seen depend upon the substituent at N-1.



(1) R= H
(2) R= CH_3
(3) R= 2'-deoxyribosyl-

(4) R= CH_3
(5) R= 2'-deoxyribosyl-
(8) R= H

(6) R= ribosyl-
(7) R= CH_3

Scheme 1

Following the method recently described by Micklitz *et al.*,[11] N-1-methylation of 5-t-butyluracil **1**[8] could be achieved in good yield. The product was fully characterised. The solubility of N-1-methyl-5-*t*-butyluracil **2** in water is rather low but we were reluctant to change the irradiation conditions previously established for the corresponding 2′-deoxynucleoside **3**[8] and therefore we decided to irradiate a stirred aqueous suspension of the compound. We knew from previous work[8] that the photoproduct would be reasonably stable under the conditions of its formation and this indeed proved to be the case. The N-1-methyl photoproduct **4** is also more water-soluble than the starting material and upon irradiation, the suspension gradually cleared to give a homogeneous solution, which after 70 hours showed a UV spectrum expected for the photoproduct. The product was purified by silica gel chromatography and could be crystallised from ethanol to give long, thin, colourless needles.

The UV spectrum of this product was essentially identical to the photoproduct previously reported which resulted from irradiation of the analogous 2′-deoxynucleoside (pH 1, λ_{max} 323 nm; ε, 7610; pH 7, λ_{max} 314 nm; ε, 13080)[8] and the NMR spectra (^1H and ^{13}C) were consistent with the structure proposed **4**. This structure was confirmed by X-ray analysis (Fig. 1) and this means that it is almost certain that the structure of the similar product **5** previously identified purely by spectral analysis was indeed correct.[8] The molecule is planar and symmetrical.

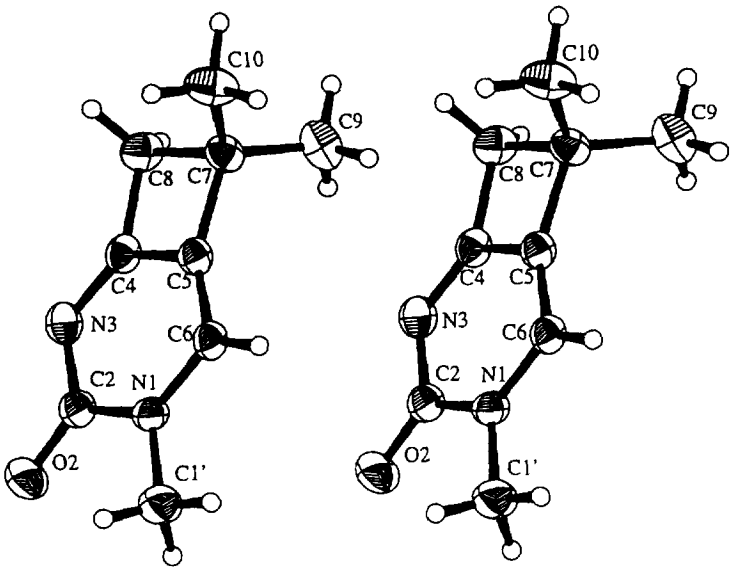


Fig. 1: X-ray crystal structure of N-1-methyl photoproduct **4**

This unusual and novel photoreaction in the pyrimidine field has already been discussed in some detail[8] and we can assume that the reaction mechanism of the reaction described here is identical and thus proceeds through a Norrish Type II mechanism where a hydrogen atom on a carbon γ- to a carbonyl group is available for abstraction. In the present case, we have an ideal substrate with 9 equivalent γ-hydrogens and other experiments (data not shown) have confirmed that a similar product is formed (in lower yield) from the 5-isopropyl-substituted pyrimidine[5] and was almost certainly also formed when 5-ethyl-2′-deoxyuridine was similarly irradiated.[12]

Thus the photoproduct **4** is a 1, 2-dihydropyrimidin-2-one. These compounds have been studied for many years, particularly as the ribonucleoside zebularine **6**, first reported in 1961,[13] is known to have an unusual spectrum of biological activity. Zebularine 5′-monophosphate inhibits thymidylate synthase[14] and zebularine itself is a potent inhibitor of cytidine deaminase[15] and possesses significant antineoplastic activity.[10]

There have been intriguing reports in the literature for many years concerning the effect of alkali on 1, 2-dihydropyrimidin-2-ones. Addition of alkali results in a bathochromic shift in the UV absorption spectrum accompanied by a huge increase in molar absorptivity. This effect was first noticed many years ago and has never been satisfactorily explained. Thus Oyen in 1969[13] reported that zebularine had λ_{max} 303-304 nm at pH 7.4, ε, 5,600 and when alkali was added, a reaction took place to give a compound with λ_{max} 315 nm and ε≈30,000. This reaction with alkali could not be achieved with 1, 2-dihydropyrimidin-2-one itself (presumably because of preferential proton N-1 abstraction) nor with the N-1 methyl derivative **7**[13] but we will show that this

latter report is incorrect. However, we can confirm that if 5-*t*-butyluracil itself is irradiated, the photoproduct so obtained (presumably **8**) is unaffected by the addition of alkali.

Later, Shugar[7] also reported an unidentified photoproduct upon irradiation of some 5-substituted uracil derivatives (we now know these photoproducts to be 1, 2-dihydropyrimidin-2-ones) and these photoproducts also showed a bathochromic shift upon addition of alkali from 307→ 348 nm and the products were referred to as PP348 (photoproducts with a λ_{max} in alkali at 348 nm). This shift was also accompanied by a dramatic rise in absorption.

More recently Marquez[9] and co-workers studied very carefully the effects caused by the addition of alkali to zebularine. They conclusively showed that the final products of such an addition of alkali are malondialdehyde **9** (λ_{max} 265 nm) and a sugar α-cyclic carbamate **10** (Fig. 2). The former compound however is clearly not responsible for the initial UV bathochromic shift seen (304→ 317 nm before finally [1 hour] undergoing a hypserchromic shift to 265 nm) and there is no explanation for the structure of the compound(s) with λ_{max} 317 nm as the compound(s) appears only momentarily after 5 minutes and the NMR spectrum of the complex mixture is uninterpretable.[9]

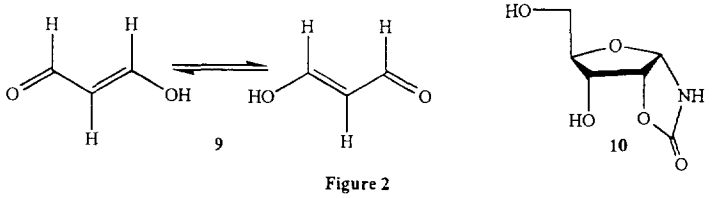


Figure 2

In the corresponding experiment in which alkali was added to our nucleoside photoproduct **5**,[8] the NMR spectrum even at 400 MHz was far too complex because of the large number of compounds of continuously varying concentration present in solution. Addition of alkali to compound **5** results in a bathochromic shift from 307→ 348 nm accompanied by a large increase in molar absorptivity which then decreases over a period of hours. We assume that the difference in bathochromic shift seen by Marquez (304→ 317 nm) and by Shugar and ourselves (307→ 348 nm) is due to the presence of the cyclobutane ring in the latter cases. As will be argued below, this increases the stability of the intermediates formed upon addition of alkali and may result either in increased conjugation of the chromophore and/ or a higher concentration of the intermediate responsible for the absorption at the longer wavelength.

Unfortunately it is not possible directly to correlate the changing UV spectra and NMR spectra. The solutions contain equilibrium mixtures of many components and the concentrations applicable to the two techniques are some three orders of magnitude different.

However, the NMR spectra of the product(s) formed by the addition of alkali to the N-methylphotoproduct **4** are much easier to interpret, despite the earlier report that alkali had no effect on a similar compound **7**.[13]

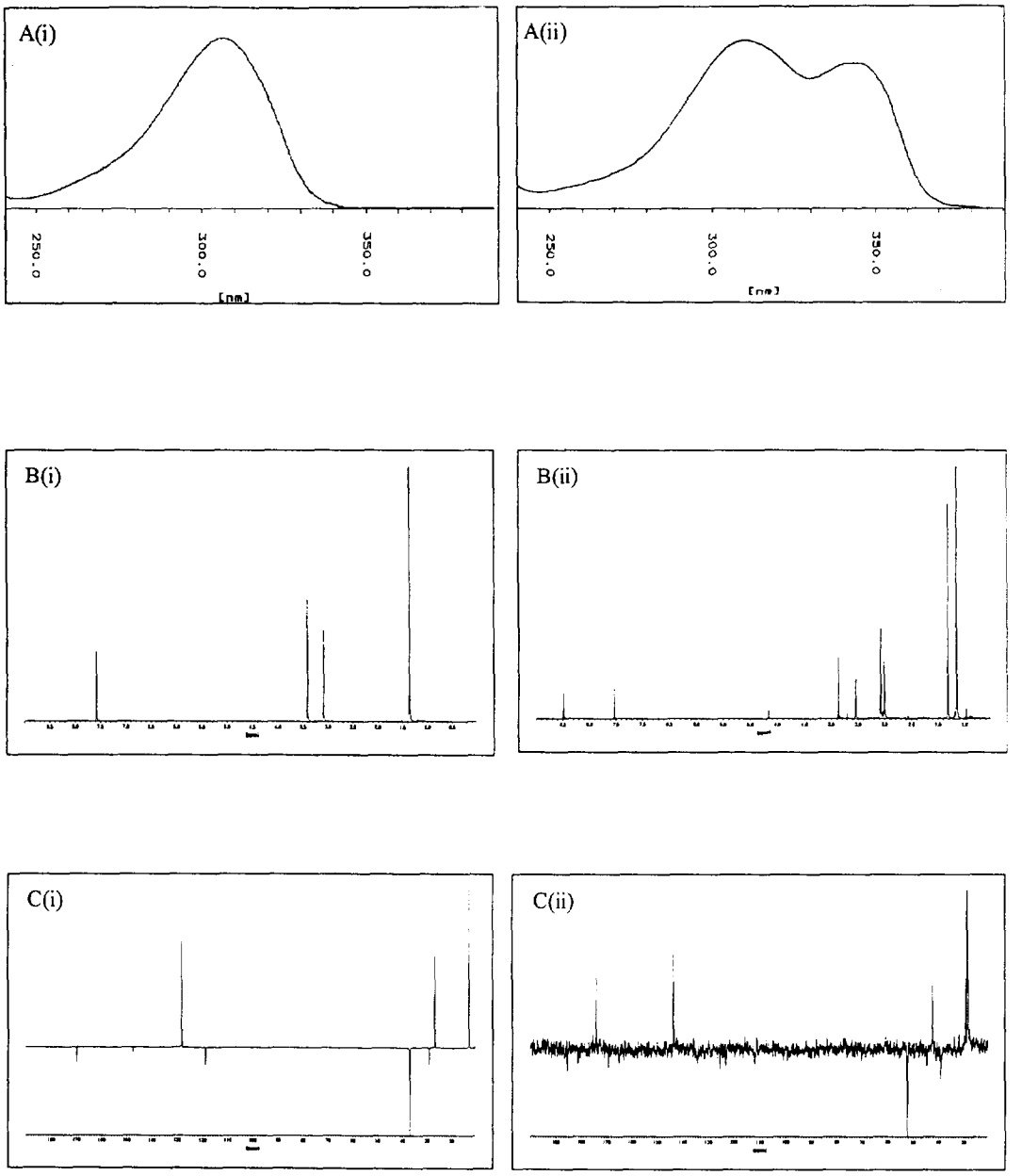
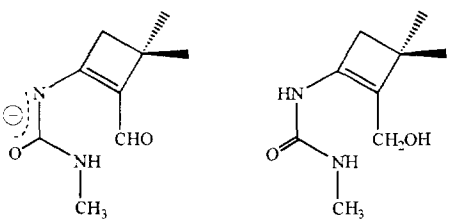


Figure 3. The effect of alkali on 1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** as recorded by (**A**) UV, (**B**) ^{1}H NMR and (**C**) ^{13}C PENDANT NMR spectroscopy. Figures denoted (i) are under neutral conditions; figures denoted (ii) are under alkaline conditions.

The ^{1}H and ^{13}C NMR spectra of the photoproduct **4** upon addition of alkali, show that there is a mixture of starting material and a new compound in the ratio of 2:3. The UV spectrum also supports this interpretation as there are new two maxima (313 and 347 nm) present.

The composition of the alkaline solution changes rapidly over a period of 2 hours but unlike the example previously studied by Marquez and co-workers, no unique and identifiable products are finally formed. This is presumably because the driving force in the case of the ribonucleoside is the production of the stable α-cyclic carbamate, whereas in the present case, no particular intermediate is significantly more stable than any other and a very complex mixture is eventually formed. However, the initial product, which is likely to correspond to the compound with a λ_{max} of 347 nm, is stable enough so that a ^{13}C NMR spectrum can be accumulated. Analysis of the ^{1}H and ^{13}C NMR spectra strongly suggests that the initial product of the reaction of alkali with the pyrimidin-2-one **4** is the open chain (hydrated) aldehyde **9** which is precisely equivalent to the intermediate suggested in the decomposition of compound **6** by Marquez which arises by attack of OH^- at C-6. Such a compound is likely to have a $\lambda_{max} \approx 348$ nm but it is difficult to quote an ε value as it is only one intermediate in an equilibrium. The ^{1}H and ^{13}C NMR spectra are entirely compatible with this structure. Thus in the ^{1}H spectrum, the main changes are the chemical shift of 'H-6' from 7.5 ppm (in **4**) to 8.5 ppm (in **11**, which corresponds to the shift expected for an α, β-unsaturated aldehyde and is similar to the corresponding proton in compound 7) and of the N-CH_3 (C-1′) from 3.4 ppm to 2.4 ppm (the latter value corresponds to the shift of the CH_3 in N-methylurea under identical conditions). In the ^{13}C spectrum, the major shifts are once again for 'C-6' (from 144 ppm to 174 ppm) and for N-CH_3 (C-1′; from 42 ppm to 29 ppm) and these chemical shifts are precisely what one would expect for a compound of structure **11** (Fig. 4). The mass spectrum of the alkaline solution also confirmed the presence of a compound with the correct mass for compound **11**.

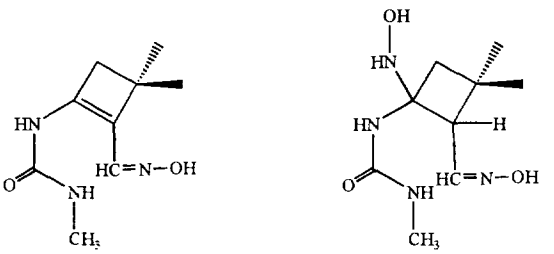


11 **12**

Figure 4

Experiments to identify a stable derivative of compound **11** were attempted and monitored by mass spectroscopy. As compound **11** is only present as an intermediate in alkaline solution, any derivative has to be produced under these conditions. Thus borohydride reduction of an alkaline solution of compound **4** resulted in the appearance immediately in the mass spectrometer of a molecular ion of 2 mass units higher than the starting material (which had vanished), and when accurately mass measured, corresponds to the product formed by the reduction of the aldehyde to the alcohol **12** (Fig. 4).

When a neutralised solution of hydroxylamine hydrochloride was added to the alkaline solution of compound **11**, mass spectroscopy showed the immediate disappearance of starting material and the appearance of a mass peak consistent with the oxime **13**. On standing for 10 minutes, a peak corresponding to a higher mass appeared which corresponds to the additional reaction of hydroxylamine across the double bond **14** (Fig. 5).[16] After 30 minutes, no sign of compounds **13** or **14** could be seen. Their identity was confirmed by accurate mass measurement.



13 14

Figure 5

EXPERIMENTAL

General: The photochemical reactions were carried out in a 400 ml immersion-well photochemical reactor with a low-pressure mercury lamp (254 nm) as the source of radiation. The NMR spectra were recorded using a Brüker AMX 400 spectrometer. All ^{13}C NMR spectra were run as ^{13}C PENDANT NMR[17] spectra. Mass spectra were obtained using a VG ZabSpec mass spectrometer. UV spectra were obtained using a Perkin Elmer UV/ VIS Spectrophotometer. Chromatography was performed on Kieselgel 60, 70-250 mesh ASTM, supplied by E. Merck AG.

The Preparation of 1-Methyl-5-t-butyluracil (**2**)

Trimethylsilylchloride (9 ml, 70 mmol) was added to a stirred solution of 5-*t*-butyluracil **1** (3.5 g, 20 mmol) in 1,1,1,3,3,3-hexadisilazane (75 ml, 390 mmol). The resulting solution was refluxed overnight under anhydrous conditions after which time the initial white solution had changed to a clear, colourless solution. The reaction mixture was cooled to 60 °C and methyl iodide (12 ml, 190 mmol) was added. The resulting solution was refluxed for 36 hours to yield a dark orange solution. Analysis by t.l.c. (EtOAc) showed the absence of the starting material and the reaction mixture was cooled to 0° C. Distilled water (100 ml) was added to the reaction mixture which resulted in the formation of a pale yellow suspension and a dark yellow oil. The water was evaporated to yield a yellow solid and the subsequent addition of methanol induced the precipitation of a white solid. This solid was collected under suction but was found to be a side product of the reaction. The filtrate was concentrated to give an orange solid and the desired product was isolated by column

chromatography (EtOAc/ hexane 4:1), followed by crystallisation with EtOAc, to yield a white solid **2** (2.15 g, 59 %), m.p. 207-210 °C, R_f (EtOAc/ hexane 4:1) 0.51; λ_{max} (pH 7) 268 nm (ε, 6810); λ_{max} (pH 14) 267 nm (ε, 6720); λ_{max} (pH 1) 270 nm (ε, 8750); δ_H (DMSO-d_6) 11.11 (1H, *s*, N^3-H), 7.31 (1H, *s*, H-6), 3.26 (3H, *s*, H-1′), 1.22 (9H, *s*, H-8, H-9, H-10); δ_C PENDANT (DMSO-d_6) 163.30 (C-4), 151.09 (C-2), 141.04 (C-6), 119.95 (C-5), 35.23 (C-1′), 32.50 (C-7), 28.74 (C-8, C-9, C-10); (EI^+) *m/z* 182 (24 %, $[M]^+$), 167 (100 %, $[C_8H_{11}N_2O_2]^+$). FOUND C, 65.69; H, 7.41; N, 17.12. $C_9H_{14}N_2O_2$ requires C, 65.83; H, 7.37; N, 17.06.

The Preparation of 1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one (**4**)

1-Methyl-5-*t*-butyluracil **2** (500 mg, 2.7 mmol) was suspended in distilled water (400 ml) to give a solution of concentration 6.9×10^{-3} mol dm^{-3}. The resulting suspension was irradiated for a total of 195 hr 15 min at room temperature, after which time the majority of the starting material had been converted to the desired photoproduct, according to the UV spectra. The apparatus was washed with methanol and the solvent was removed by evaporation to yield a yellow solid. Analysis by t.l.c. (EtOAc/ MeOH 9:1) showed the presence of four products. The desired product was isolated by column chromatography (EtOAc/ MeOH 9:1) to yield a pale yellow solid **4** (195mg, 43%), m.p. 188-91 °C (decomp.), R_f (EtOAc/ MeOH 9:1) 0.26; λ_{max} (pH 7) 307 nm (ε, 5000); λ_{max} (pH 14) 344 nm (ε, 4540), 310 nm (ε, 5280); λ_{max} (pH 1) 323 nm (ε, 7280); δ_H (DMSO-d_6) 7.77 (1H, *s*, H-6), 3.33 (8H, *2 × s*, H-1′+ H_2O), 3.06 (2H, *s*, H-8), 1.39 (6H, *s*, [H-9, H-10]); δ_H (DMSO-d_6 + D_2O) 7.69 (1H, *s*, H-6), 3.89 (H_2O), 3.33 (3H, *s*, H-1′), 3.05 (2H, *s*, H-8), 1.37 (6H, *s*, [H-9, H-10]); δ_C PENDANT (DMSO-d_6) 180.22 (C-4), 140.08 (C-6), 127.87 (C-5), 49.92 (C-8), 40.82 (C-7), 38.79 (C-1′), 26.85 ([C-9, C-10]), C-2 unresolved; (EI^-) *m/z* 164 (92 %, $[M]^+$), 149 (43 %, $[M\text{-}CH_3]^+$), 135 (74 %, $[M\text{-}(NCH_3)]^+$); Accurate mass $C_9H_{12}N_2O$ requires 164.0950. Observed 164.0952. FOUND C, 59.31; H, 7.70; N, 15.30. $C_9H_{12}N_2O$ requires C, 59.32; H, 7.74; N, 15.37.

The Treatment of 1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one (**4**) *with Alkali*
^1H NMR Spectra

1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** (15 mg, 0.1 mmol) was dissolved in D_2O (0.5 ml) to give a solution of concentration of 0.18 mol dm^{-3}. A ^1H NMR spectrum of this solution was recorded; δ_H (D_2O) 7.58 (1H, *s*, H-6), 3.42 (3H, *s*, H-1′), 3.09 (2H, *s*, H-8), 1.36 (6H, *s*, [H-9, H-10]);). NaOD (40%, 0.05ml) was added to the solution and ^1H NMR spectra were recorded at regular time intervals for up to 60 hours; δ_H (D_2O + NaOD, 1 minute) 8.48 (1H, *s*, H-6), **7.52 (1H, *s*, H-6)**, **3.37 (1H, *s*, H-1′)**, **3.04 (2H, *s*, H-8)**, 2.58 (3H, *s*, H-1′), 2.50 (2H, *s*, H-8), **1.32 (6H, *s*, [H-9, H-10])**, 1.15 (6H, *s*, [H-9, H-10]).

Starting material appears in bold type; photoproduct appears in plain type.

^{13}C NMR Spectra

1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** (15 mg, 0.1 mmol) was dissolved in D_2O (0.5 ml) to give a solution of concentration of 0.18 mol dm^{-3}. A ^{13}C NMR spectrum of this solution was recorded; δ_C (D_2O) 185.28 (C-4), 165.00 (C-2), 143.56 (C-6), 134.12 (C-5), 52.30 (C-8), 44.39 (C-7), 42.22 (C-1′), 28.17 (C-9, C-10). NaOD (40 %, 0.05 ml) was added to the solution and each ^{13}C NMR spectra was accumulated over a period of 15 minutes. These spectra were recorded for 90 minutes after the addition of alkali; δ_C (D_2O + NaOD, 90 minute) **185.23 (C-4)**, 181.25 (C-4), **173.86 (C-6)**, 169.17 (C-2), **168.86 (C-2)**, 143.51 (C-6), **134.07 (C-5)**, 125.28 (C-5), 56.04 (C-8), **52.25 (C-8)**, **44.34 (C-7)**, 38.92 (C-7), **42.18 (C-1′)**, 29.00 (C-1′), 28.46 (C-9, C-10), **28.15 (C-9, C-10)**.

Starting material appears in bold type; photoproduct appears in plain type.

Mass Spectroscopy Spectrum

1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** (3.1 mg, 0.1 mmol) was dissolved in aqueous NaOH (40 μl, 1 M) to give a solution of concentration 4.7×10^{-1} mol dm^{-3}. A mass spectrum was recorded using thioglycerol as the matrix; (FAB) *m/z* 187 (100 %, [**4** + Na^+]), 205 (21 %, $[M + Na]^+$, 227 (20 %, $[M + 2Na]^+$). Accurate mass $C_9H_{14}N_2O_2Na$ requires 205.0953. Observed 205.0951.

The Reduction of an Alkaline Solution of 1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one (4) using Sodium Borohydride

1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** (3.1 mg, 0.02 mmol) was dissolved in aqueous NaOH (40 μl, 1 M) to give a solution of concentration of 4.7×10^{-1} mol dm^{-3}. After 5 minutes, sodium borohydride (1 mg, 0.02 mmol) was added to the solution and the solution was mixed well. A mass spectrum of this solution was recorded after 5 minutes; (FAB) *m/z* 207 (48 %, $[M + Na]^-$), 189 (86 %, $[M - H_2O + Na]^-$). Another mass spectrum was recorded 10 minutes later; (FAB) *m/z* 209 (22 %, $[M-2H + Na]^-$), 189 (77 %, $[M - H_2O + Na]^-$). Accurate mass $C_9H_{16}N_2O_2Na$ requires 207.1109. Observed 207.1105.

The Reaction of an Alkaline Solution of 1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one (4) with Hydroxylamine

1-Methyl-7,7-dimethyl-2, 4-diaza-bicyclo-[4.2.0]-octa-1, 5-diene-3-one **4** (3.1 mg, 0.02 mmol) was dissolved in aqueous NaOH (40 μl, 1 M) to give a solution of concentration of 4.7×10^{-1} mol dm^{-3}. After 5 minutes, an aqueous hydroxylamine solution (40 μl, 0.02 mmol) was added to the solution and the solution was mixed well. A mass spectrum of this solution was recorded after 5 minutes; (FAB) *m/z* 253 (55 %, $[M + NH_2OH + Na]^+$), 220 (100 %, $[M + Na]^+$). Accurate mass $C_9H_{15}N_3O_2Na$ requires 220.1062. Observed 220.1064.

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