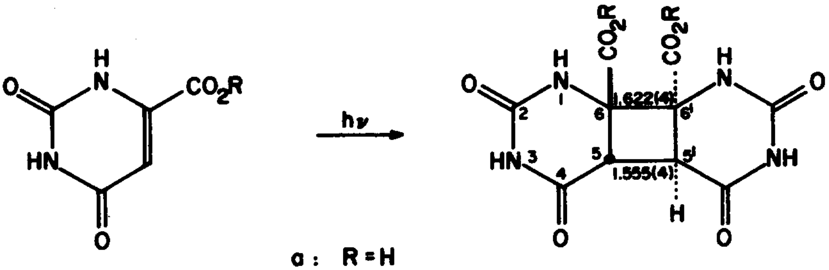
Tetrahedron Letters No.14, pp. 947-950, 1971. Pergamon Press. Printed in Great Britain.

PREPARATION AND STRUCTURE OF PHOTODIMERS OF METHYL OROTATE AND OROTIC ACID

G. I. Birnbaum[*], J. M. Dunston[#], and A. G. Szabo[†]
National Research Council of Canada, Ottawa, Ontario, Canada.

(Received in USA 1 March 1971; received in UK for publication 16 March 1971)

In view of their biological significance, photoproducts of nucleic acids and related substances have received considerable attention in recent years. X-ray analyses (1) have revealed their structures and have provided detailed information about their geometry. Although the photochemistry of orotic acid 1a and its derivatives has been studied by several people (2), the structure of the major photoproduct has been assigned only indirectly as a cyclobutane dimer. Recent mechanistic studies on the photochemistry of orotic acid and its methyl ester 1b (3,4) make the absolute characterization of the photoproducts desirable. In this communication we report the photochemical preparation and isolation of the photoproduct of methyl orotate and its identification by X-ray crystallography as the *trans-syn* cyclobutane photodimer 2b. The photodimer of orotic acid was interrelated with 2b and was shown to have the same stereochemistry.



1 a: R = H
b: R = CH_3 2

Aqueous solutions (2ℓ) of methyl orotate (5) (1.7×10^{-3} M) were irradiated (6) for fifteen hours after which time the ultraviolet absorption spectrum of an aliquot indicated only end absorption. Thin layer chromatography (Cellulose MN 300 HR, propanol/water, 1:1) indicated only one product

---

[*] Biochemistry Laboratory, NRCC.
[#] Visiting Scientist, 1969, Division of Biology, NRCC.
[†] Division of Biology, NRCC.

and the complete absence of methyl orotate. Evaporation of the colourless solution provided a white powder which gave colourless crystals (435 mg, 73%) after recrystallization from water (7). On heating, the crystals began to melt at 195°. At 210° they changed into smaller crystals which remelted at 244-245°. Besides the melting point these new crystals were identified as methyl orotate by comparison of ultraviolet and infrared spectra. When an aqueous solution of the crystalline photoproduct was irradiated without a filter, methyl orotate was quickly regenerated. The above evidence, taken together with the spectral data of the photoproduct (ν_{max} (nujol) 3355, 3310, 1730, 1708 cm^{-1}; n.m.r. (8) ($DMSO-d_6$) σ=3.70 (s, 4H), σ=8.00 (s, 1H), σ=10.01 (s, 1H) p.p.m.), indicated that the photoproduct was a cyclobutane dimer. The infrared spectrum no longer included bands at 1746, 1685, and 1505 cm^{-1}, and this could be attributed to the loss of the double bond from methyl orotate. The n.m.r. spectrum was consistent with a cyclobutane structure, the band at σ=3.70 p.p.m. being assigned to a coincidence of the proton signals from the carbomethoxy methyl and cyclobutane hydrogens (9). However, neither the above evidence nor the C^{13}-H satellite proton spectrum enabled us to decide the stereochemistry of the photodimer. In order to resolve this question the substance was subjected to X-ray analysis.

The photodimer crystallized as the monohydrate. Precession photographs were taken and systematically absent reflections indicated two possible monoclinic space groups, one of them non-centrosymmetric (Cc) and the other centrosymmetric (C2/c). The crystal had the following cell dimensions: a=14.024, b=6.722, c=17.006Å; β=105.11°. The data were measured with a four-circle automatic diffractometer fitted with a Cu target tube. The moving-crystal, moving-counter ($\Theta/2\Theta$ scan) method was employed. The first crystal deteriorated slowly in the X-ray beam and half-way through the data collection it was replaced by another one. Of the 1308 accessible reflections ($2\Theta \leq 130°$) 1234 had observable intensities. The structure factors were normalized and the statistical distribution of the E-values was computed. The results indicated clearly that the space group was centrosymmetric. Since the space group C2/c has 8 equivalent positions, this implied, in view of the crystal density, that the asymmetric unit was one-half of the dimer molecule. The structure was solved by the symbolic addition method (10). Two reflections [7 5 $\bar{5}$ (E=3.71) and 7 1 $\overline{14}$ (E=2.89)] were chosen to define the origin and were given positive signs. A computer program (written by S.R. Hall) was used which assigns symbols to 4 suitable reflections and attempts to determine their signs from successive application of the $\sum_2$ relationship. The search for triplets which covered 101 reflections with E≥1.8, yielded the signs of only 2 symbols, resulting in 29 signed and 72 unsigned reflections. This left 4 possible combinations of signs (++,+-,-+,--) for the two undetermined symbols. When the first of these combinations was assumed and 156 reflections with E≥1.5 were included only 5 reflections remained unsigned. An E-map was calculated on the basis of these 151 reflections and it revealed the positions of all non-hydrogen atoms, including that of the water oxygen. Least-squares refinement of the atomic and anisotropic thermal parameters has led to a current value of the agreement factor, R=6.2%. The refinement is being continued.

The structure of the dimer, as revealed by this X-ray analysis, is that of the *trans-syn* isomer (2b). Although X-ray analyses of the other three possible isomers of pyrimidine photodimers (*cis-syn*, *cis-anti*, *trans-anti*) have been published (1), this is the first such determination of a *trans-syn* isomer. The twofold axis of rotation which passes through the molecule bisects the two interpyrimidine bonds of the cyclobutane ring, making the two halves of the dimer equivalent. The cyclobutane ring is puckered but considerably less so than other non-planar cyclobutanes; the dihedral angle is 169.5°. Consequently, the pyrimidine rings are also near-planar with approximate half-chair conformation. The largest displacements from the "best" plane through this ring are 0.06Å for C(6) and 0.05Å for C(5). The two interpyrimidine bonds are markedly different from each other and the C(6)-C(6') bond is unusually long. This is due to the fact that both carbon atoms are fully substituted and a very close contact (2.774Å) exists between N(1) and the carbon atom attached to C(6'). This long bond accounts for the ease with which this dimer splits into monomers by the action of radiation and/or elevated temperature. The C(5)-C(5') bond as well as other bond lengths are normal for this class of compounds.

The dimer molecules are hydrogen-bonded to each other *via* pairs of N(1)····O(4) bonds. They are also linked *via* the solvent water molecule which donates one proton to O(2) and to O(4) in two different dimer molecules (bifurcated hydrogen bond) and another proton to the carbonyl oxygen of the carbomethoxy group in a third molecule. This hydrogen bond system may account for the near-planarity of the cyclobutane and the pyrimidine rings. Full details of the X-ray analysis will be published elsewhere (by G.I.B.).

The absolute assignment of the photoproduct of methyl orotate as 2b made it possible for us to determine the structure of the orotic acid photodimer. When the latter (11) was treated with methanolic diazomethane, the resultant product was shown to be identical with the photodimer 2b. The infrared and n.m.r. spectra of this methylated derivative were superimposable on those of 2b, and it exhibited identical m.p. and t.l.c. behaviour as 2b. Therefore, the photodimer of orotic acid has the structure 2a and not the *trans-anti* structure as had been suggested earlier (12).

Since methyl orotate forms only one detectable photoproduct, the *trans-syn* cyclobutane dimer, the difference in lifetimes of the triplet excited states of methyl orotate (287 μs) and orotic acid (78 μs) (4) must reflect a variation in the chemistry of this state for the two molecules. Our own preliminary work on the photochemistry of orotic acid supports this conclusion

(11). In the past (2) orotic acid had been used as a model system to study the kinetics and mechanism of the photodimerization of pyrimidines. The unique photoreactivity of methyl orotate suggests its use as a more reliable model. The formation of only one cyclobutane dimer from methyl orotate is likely governed by thermodynamic rather than kinetic control. The most stable diradical intermediate would be the one resulting from first bond formation between the C(5) atoms of an excited and a ground state molecule, thereby leading to a head-to-head (*syn*) product (cf. ref. 13).

REFERENCES

(1) See E. Adman and L.H. Jensen, *Acta Cryst.*, **B26**, 1326 (1970) for a recent review.

(2) (a) W. Berends, *J. Chim. Phys.*, **58**, 1034 (1961); (b) E. Sztumpf and D. Shugar, *Photochem. Photobiol.*, **4**, 719 (1965); (c) M. Charlier, C. Hélène, and M. Dourlent, *J. Chim. Phys.*, **66**, 700 (1969).

(3) D.W. Whillans and H.E. Johns, *Photochem. Photobiol.*, **9**, 323 (1969).

(4) R.W. Yip, W.D. Riddell, and A.G. Szabo, *Can. J. Chem.*, **48**, 987 (1970).

(5) Preparation and spectral properties are recorded in ref. 4.

(6) Irradiations were conducted with a Hanovia 450W medium pressure lamp using a Pyrex filter. Nitrogen was bubbled through the solution during the irradiation.

(7) Satisfactory analytical and molecular weight determinations were obtained for this product.

(8) N.m.r. spectra were performed on a Varian HA-100A spectrometer; the chemical shifts are recorded relative to TMS as an internal standard.

(9) D.L. Wulff and G. Fraenkel, *Biochim. Biophys. Acta*, **51**, 332 (1961).

(10) J. Karle and I.L. Karle, *Acta Cryst.*, **21**, 849 (1966).

(11) This photodimer was prepared, isolated and characterized as a cyclobutane dimer in the same manner as that described for methyl orotate. The t.l.c. indicated that the isolated dimer was the major product although an additional product was formed.

(12) R. Lisewski and K.L. Wierzchowski, *Photochem. Photobiol.*, **11**, 327 (1970).

(13) P. Yates and D.J. MacGregor, *Tetrahedron Letters*, 453, (1969).