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Photochemical Reactivity of 3-Ethoxycarbonylfurocoumarin[1)]

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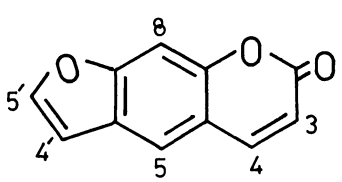
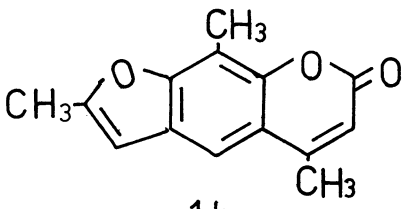
Photochemical reactivity of 3-ethoxycarbonylfurocoumarin is studied. Upon irradiation, 3-ethoxycarbonylfurocoumarin forms a cyclobutane with a variety of alkenes at the 3,4-double bond in solution, whereas a similar cyclobutane is formed at the 4',5'-double bond in DNA. The different site-selectivity in solution from DNA is explained by assuming the involvement of the triplet excited state.

Furocoumarins have been successfully used in the treatment of skin diseases such as psoriasis and vitiligo.[2)] The biological effect of furocoumarins is ascribed to their photochemical reactivity toward DNA. Upon irradiation in DNA, two reactive sites of furocoumarins yield cyclobutanes in their reaction with thymine residues, resulting in the cross-linking of two strands of DNA. Among two reactive sites, the 4',5'-double bond shows a higher reactivity toward the double bond of thymine residues than the 3,4-double bond in typical furocoumarins such as 1a, 1b, or 1c. Therefore, the first step of cross-linking of DNA is the [2+2] cycloaddition at the 4',5'-double bond of furocoumarins.[2)] On the contrary, similar cycloaddition reaction takes place exclusively at the 3,4-double bond of furocoumarins upon irradiation in solution.[3)] Such a remarkable difference in reactivities of two reactive sites of furocoumarins in different environments has not been explained clearly.

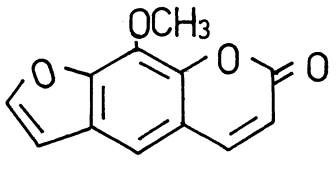
Here, the photochemical reactivity of furocoumarin 3-carboxylic acid ester is studied in order to gain a better understanding of the biological effect of furocoumarins.[4)] The introduction of ester moiety at the 3-position was reported



1a

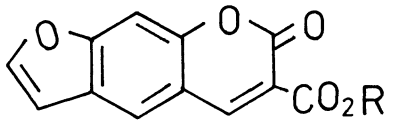


1b



1c

to reduce the reactivity of the 3,4-double bond of furocoumarin dramatically in DNA.[5] Because of the failure to detect the photo-cycloaddition product at the 3,4-double bond in DNA, 3-ethoxycarbonylfurocoumarin 2a, for example, is sometimes called a monofunctional furocoumarin. The effect of 3-ethoxycarbonyl group to reduce the reactivity of the 3,4-double bond has been explained due to its electronic and/or steric effect.

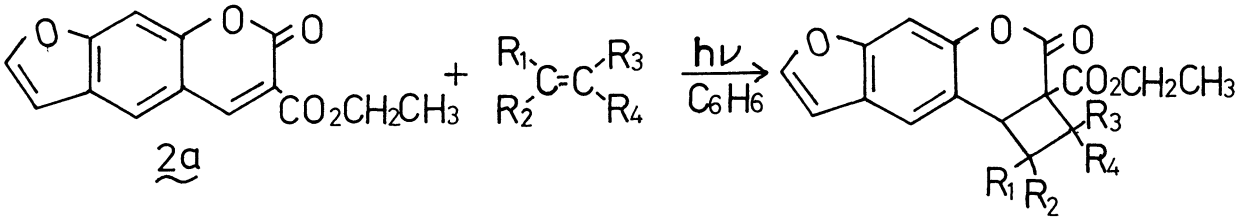


2a (R = CH_2CH_3)

Interestingly, the photochemical reactivity of 3-ethoxycarbonylfurocoumarin 2a in solution was found to be quite different from that reported in DNA. When 2a (1.6 mM) is irradiated in the presence of 2,3-dimethyl-2-butene (9.5 mM) in benzene as the solvent, a smooth reaction affords a cyclobutane adduct in 55% yield through [2+2] cycloaddition. Judging from the detailed analysis of the spectroscopic data, the cyclobutane is concluded to be formed at the 3,4-double bond of 2a (see the structure 3a).[6] The photochemical reactivity of the 3,4-double bond of 2a has never been reported. Similar cyclobutanes formed at the 3,4-double bond of 2a are isolated in the photochemical reaction of 3-ethoxycarbonylfurocoumarin 2a with a variety of alkenes. They are 2-methylpropene (yield of the corresponding cyclobutane 3b: 73%), 2-ethyl-1-butene (3c: 70%), ethyl vinyl ether (3d: 85%), styrene (3e: 71%), and p-methylstyrene (3f: 62%).[7,8] The observed reactivity of 2a in solution shows a remarkable contrast with the reported reactivity of 2a intercalated in DNA.

Thus, in solution the reactivity of the 3,4-double bond of furocoumarins is concluded higher than that of the 4',5'-double bond regardless of the substituents.

The formation of a cyclobutane through the reaction at the 3,4-double bond of furocoumarin 1a with 2,3-dimethyl-2-butene was found to be linearly



2a

	R_1	R_2	R_3	R_4
3a:	CH_3	CH_3	CH_3	CH_3
b:	CH_3	CH_3	H	H
c:	CH_3CH_2	CH_3CH_2	H	H
d:	CH_3CH_2O	H	H	H
e:	C_6H_5	H	H	H
f:	p-$CH_3C_6H_4$	H	H	H

quenched with the addition of naphthalene as the triplet quencher.[9] This result suggests the cyclobutane is formed at the 3,4-double bond via the triplet excited state of furocoumarins. The efficiency of triplet formation of 3-ethoxycarbonylfurocoumarin 2a (0.30 in benzene) was reported much higher than that of furocoumarin 1a (0.035 in benzene) or 8-methoxyfurocoumarin 1c (0.011 in benzene).[10] Thus, the observed efficient formation of cyclobutanes at the 3,4-double bond of 2a can be explained by assuming an efficient formation of the triplet excited state of 2a in benzene. In summary, the photochemical reactivity of the 3,4-double bond of furocoumarins in solution can be concluded intrinsically higher than the 4',5'-double bond in their triplet excited state, regardless of their substituents. The steric effect of the ester moiety on the reactivity of the 3,4-double bond of furocoumarin is currently being studied by introducing different alkyl groups in size.

However, the present result would suggest that non-bonded interactions prior to the photochemical events play rather important role to determine the site-selectivity of furocoumarins intercalated in DNA.[11]

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References

1) This paper is dedicated to the memory of the late Professor E.T. Kaiser, Rockefeller University.

2) J.A. Parrish, "Phototherapy of Psoriasis and Other Skin Diseases," in "The Science of Photomedicine" ed by J.D. Regan and J.A. Parrish, Plenum Press, New York (1982), p.511; C.D. Cimino, H.B. Gamper, S.T. Isaacs, and J.E. Hearst, Ann. Rev. Biochem., 54, 1151 (1985) and the references cited therein.

3) T. Otsuki, Chem. Lett., 1987, 453.

4) Synthesis of 3-ethoxycarbonylfurocoumarin 2a was reported: L.R. Worden, K.D. Kaufman, J.A. Weis, and T.K. Schaaf, J. Org. Chem., 34, 2311 (1969).

5) D. Averbeck, E. Moustacchi, and E. Bisagni, Biochim. Biophys. Acta, 518, 464 (1978); J. Cadet, L. Voisturiez, F. Gaboriau, P. Vigny, and S. Della Negra, Photochem. Photobiol., 37, 363 (1983).

6) Mp 120 - 124 °C. Exact mass, m/z Found 342.1480: Calcd for $C_{20}H_{22}O_5$ 342.1467. ^{1}H-NMR($CDCl_3$):δ; 0.76 ppm (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.2 - 1.5 (m, 3H, CH_3 in CH_2CH_3), 4.02 (m, 1H, H_4), 4.1 - 4.3 (m, 2H, CH_2 in CH_2CH_3), 6.72 (dd, 1H, H_4' J = 2.0, 0.6 Hz), 7.18 (br s, 1H, H_5), 7.27 (s, 1H, H_8), 7.60 (d, 1H, H_5', J = 2.0 Hz). ^{13}C-NMR($CDCl_3$):δ; 14.2, 20.8, 21.4, 23.0, 24.0, 43.6, 45.8, 50.7, 55.2, 62.1, 99.8, 106.1, 114.6, 119.8, 124.4, 145.8, 149.3, 154.2, 163.6, 167.7 ppm. IR(KBr): 2973, 2932, 1762, 1726, 1461, 1375, 1244, 1220, 1194, 1165, 1131, 1029 cm^{-1}. UV max($CHCl_3$): 297.8 nm (ε: 3.7 x 10^2), 287.3 (4.9 x 10^2), 258.3 (1.3 x 10^3). The UV spectrum clearly indicates that the cyclobutane

is formed through the reaction at the 3,4-double bond. Otherwise, the intact conjugated carbonyl gives a strong absorption around 330 nm. Cf., Refs. 2 and 3.

7) All isolated cyclobutanes gave satisfactory spectral data including exact mass.

8) The regiochemistry of cyclobutanes has been assigned based on the detailed analysis of ^{1}H-NMR spectra. The stereochemistry of cyclobutanes is tentatively estimated trans by a careful comparison of the ^{1}H-NMR signals. Since the chemical shift of the methyl group in the cyclobutane 3f (2.36 ppm) is essentially the same to that of the methyl group of p-methylstyrene as the starting alkene (2.34 ppm), the methyl group in 3f can be concluded not to be influenced by the anisotropy of furocoumarin moiety in the cyclobutane, that is, trans. The further study by X-ray will be planned to confirm the stereochemistry. On the other hand, in DNA cis-cyclobutanes are formed presumably as a consequence of non-bonded interactions prior to the photochemical events. Cf., Ref. 2.

9) The triplet energy of furocoumarins was estimated ca. 63 kcal/mol: W.W. Mantulin and P-S. Song, J. Am. Chem. Soc., 95, 5122 (1973). From the slope of the Stern-Volmer plot, $K_q\tau$ is estimated 2.4×10^2 M^{-1}.

10) R.V. Bensasson, E.J. Land, and C. Salet, Photochem. Photobiol., 27, 273 (1978); J.C. Ronford-Haret, D. Averbeck, R.V. Bensasson, E. Bisagni, and E.J. Land, ibid., 35, 479 (1982).

11) Such non-bonded interactions in DNA would result in the change of the mechanism of photoaddition reaction of furocoumarins in DNA from in solution. Since addition of DNA to an aqueous solution of furocoumarins decreases their fluorescence intensity, the different site-selectivity in DNA can possibly be explained by assuming the intervention of electron transfer process via the singlet excited furocoumarins or their exciplex. However, the involvement of the singlet excited state or the exciplex of furocoumarins in DNA has not yet been demonstrated unambiguously. Cf. P.C. Beaumont, B.J. Parsons, S. Navaratnam, G.O. Phillips, and J.C. Allen, Biochim. Biophys. Acta, 608, 259 (1980); R.V. Bensasson, NATO Adv. Study Inst., Series A, 85, 241 (1985).

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