consideration of these chemical and physical data, the major cycloadduct was conclusively designated as III.[8]

The formation of III may adequately be explained in terms of photochemical rearrangement of the initially-produced cycloadduct (II) through a biradical intermediate (IX) or the likes, which seems similar to the photochemical behavior of $\beta,\gamma$-unsaturated ketone system.[9] This was also verified by the fact that irradiation of II in dioxane actually afforded III, accompanied with an unidentified product.

In contrast to the above case, heating of I and dimethyl acetylenedicarboxylate at 100° did not yield II nor III; a considerable amount of I was recovered and any other 1:1 cycloadduct was not successful to be characterized.

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8) We also can not deny the possibility of an alternative formula (X) which, however, based on its mechanistic rationalization, is very unlikely. In addition, precise analysis, including decoupling study, of the signals at 4.84 and 7.76 ppm in the NMR spectrum of III did not exhibit any mutual coupling, also ruling out the possibility of X.

9) G.C. Schenck and R. Steinmetz, *Chem. Ber.*, **96**, 520 (1963); D.I. Schuster, M. Axelrod, and J. Auerbach, *Tetrahedron Letters*, 1911 (1963); E.F. Keifer and D.A. Carlson, *Tetrahedron Letters*, 1617 (1967); D.E. Bays and R.C. Cookson, *J. Chem. Soc.*, Sect. B, 226 (1967); L.A. Paquette and R.F. Eizenber, *J. Am. Chem. Soc.*, **89**, 6205 (1967); J.K. Crandall, J.P. Arrington, *ibid.*, **89**, 6208 (1967).

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## Photo-induced Oxygenation of Hydrocarbons by Pyridazine N-oxides[1]

Recently, many works concerning to the photolyses of aromatic amine N-oxides have been reported.[2] However, none of the N-oxides so far examined photochemically contains an internal azoxy function (I) in their structures such as pyridazine, cinnoline, and phthalazine. This fact together with an interesting photochemical intramolecular rearrangement of oxygen atom in azoxybenzene[3] has prompted us to examine the photochemical behavior of several pyridazine N-oxides (IIa[4] and IIb[5]).

As the result, we have found that the photolyses of these N-oxides in benzene solution resulted in the formation of the corresponding pyridazines (IIIa,b) and phenol as the main

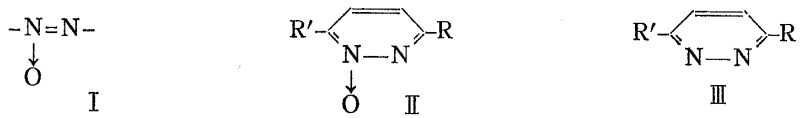
1) This paper forms part IX of a series entitled to "Syntheses of Pyridazine Derivatives". For previous paper, see, *Chem. Pharm. Bull.* (Tokyo), **15**, 2000 (1967).

2) C. Kaneko, Sa. Yamada, I. Yokoe, and M. Ishikawa, *Tetrahedron Letters*, 1873 (1967); J. Streith, H.K. Darrah, and M. Weil, *ibid.*, 5555 (1966); O. Buchardt, B. Jensen, and I.K. Larsen, *Acta Chem. Scand.*, **21**, 1841 (1967); N. Ikekawa and Y. Homma, *Tetrahedron Letters*, 1197 (1967).

3) G.M. Badger and R.G. Buttery, *J. Chem. Soc.*, 2243 (1954); R. Tanikaga, K. Maruyama, R. Goto, and A. Kaji, *Tetrahedron Letters*, 5925 (1966).

4) T. Itai and S. Natsume, *Chem. Pharm. Bull.* (Tokyo), **11**, 83 (1963).

5) H. Kano, M. Ogata, H. Watanabe, and I. Ishizuka, *Chem. Pharm. Bull.* (Tokyo), **9**, 1017 (1963); T. Nakagome, *Yakugaku Zasshi*, **82**, 249 (1962).



I II III

a: R=R′=H
b: R=H, R′=$CH_3$

products in the moderate yields (30—40%) and also found that the same products were obtained when these N–oxides and benzene (5—10 mole equivalent to the N–oxide used) were irradiated in a large excess of dichloromethane. This remarkable oxidation of an aromatic hydrocarbon to its corresponding oxygenated product by this procedure can also be effected to the saturated hydrocarbons such as cyclohexane. In this communication, the authors will report some of these experiments.

Pyridazine 1–oxide (IIa or IIb) in benzene was irradiated by high pressure mercury lamp[6] with pyrex filter under nitrogen atmosphere until all of the starting N–oxide was consumed. From this solution, the deoxygenated product[7] (the corresponding pyridazine, IIIa or IIIb) and phenol[8] were obtained in *ca.* 35—40% yields.[9]

Similarly, irradiation of (II) in toluene resulted in the formation of the corresponding pyridazines (IIIa,b) and a mixture of cresols in almost same yields as above. It should be noteworthy in this case that benzyl alcohol was not obtained.

The photolysis of (II) and their 5—10 mole equivalent amounts of benzene or toluene in dichloromethane also gave the same products as above, though the yield were slightly lower (30—35%). By this procedure, naphthalene was also oxygenated to a mixture of naphthols in total yield of 20—30% (the ratio of $\alpha$– and $\beta$–naphthol was about 1:0.8).

Similarly, irradiation of pyridazine N–oxide (IIa) in dichloromethane in the presence of 5—10 mole equivalent of cyclohexane gave rise to cyclohexanol in *ca.* 30% yield together with the deoxygenated product (IIIa). The formation of cyclohexanol clearly demonstrated that the oxygen atom liberated from pyridazine N–oxides (II) under these conditions attacked to the carbon–hydrogen bond. At present, however, it could not be concluded that the formation of phenol from benzene had been resulted by insertion of an oxygen atom to the carbon–hydrogen bonds. Because in this case, an alternative route may be considered, in which the addition of an oxygen atom to the double bond to yield benzene oxide[10] which may then rearrange to phenol.

Finally the mixture of pyridazine 1–oxide (IIa) and 1:1 mole ratio benzene–cyclohexane (each is 5 mole equivalent to IIa) was irradiated in dichloromethane. The amount of phenol formed was about twice as that of cyclohexanol.

More extensive study on this novel photochemical oxidation reactions is underway in our laboratory and the result will be reported in a near future.

The reaction products were identified by gas chromatography and also as their derivatives, namely, phenol as its benzoate (mp 68—69°), cyclohexanol as its phenylurethane (mp 81—82°), and pyridazines as their picrates (IIIa: mp 168—169°, IIIb: mp 145—146°).

6) High–pressure mercury lamp (200 W, Osawa Denki Co., Japan) was used as a light source throughout the following experiments.
7) The photochemical deoxygenation of aromatic amine N–oxides has been reported by several workers. For example, see: T. Kosuge, K. Adachi, *et al.*, *Yakugaku Zasshi*, **85**, 66 (1965); M. Ishikawa, Sa. Yamada, H. Hotta, C. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **14**, 1102 (1966).
8) During the preparation of the present paper, a paper by J. Streith, *et al.* appeared (*Chem. Comm.*, 979 (1967)), in which they reported the formation of phenol by the irradiation of pyridine N–oxides in benzene.
9) This and other irradiation experiments alway yield the decomposition products of (II). The details will be reported separately. *cf.* M. Ogata and H. Kano, *Chem. Comm.*, 1176 (1967).
10) E. Vogel and H. Günther, *Angew. Chem. Intern . Ed. Engl.*, **6**, 385 (1967).

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# Occurrence of a New Active Peptides on Smooth Muscle and Bradykinin in the Skin of *Rana nigromaculata* Hallowell

Similar to some amphibian skin,[1] methanol extracts of wet skin of *Rana nigromaculata*, a Japanese amphibian, was found to contain some active peptides which possess a potent stimulant action on isolated rat uterus.

The present communication describes the purification and identification of these active peptides. Separatory method of the peptides is shown in Chart 1.

**Characterization and Structure of the Peptide I**——The peptide gave a single dimethylaminonaphthalenesulfonyl (DNS) derivative by thin-layer chromatography on silica gel H. The peptide was stable against tryptic digestion, but cleaved with chymotrypsin to yield three fragments including free arginine. DNS-valine was obtained as the N-terminus when the DNS-peptide I was hydrolyzed with 6 N HCl at 105° for 24 hours, and following amino acids were also determined by amino acid analyzer: $Thr_1$, $Pro_3$, $Gly_1$, $Phe_2$, $Arg_1$. From the results of Edman degradation combined with DNS method,[2] which was performed to the last sequence, it was concluded that the structure of the peptide I was $val^1$–$thr^6$–bradykinin (Val–Pro–Pro–Gly–Phe–Thr–Pro–Phe–Arg). Relative contracting activity of $val^1$–$thr^6$–bradykinin on isolated rat uterus was about 1/5 to that of bradykinin.

**Characterization of the Peptide II**——This peptide was identified with bradykinin by the evidence described below. DNS derivative of the peptide showed the same *Rf* value as DNS bradykinin on a thin-layer of silica gel H. N-terminal amino acid was arginine. Chromatographic behaviors of the DNS-peptide II after the treatment with trypsin or chymotrypsin were identical to that of DNS-bradykinin. Amino acid composition was also identical to bradykinin.

Complete details will be published elsewhere. Total synthesis and further pharmacological investigation of $val^1$–$thr^6$–bradykinin are in progress.

1) V. Erspamer and A. Anastasi, "Hypotensive Peptides," ed. by E.G. Erdös, N. Back, and F. Sicuteri, Springer-Verlag, New York, Inc., 1966, pp. 63—75.
2) W.R. Gray and B.S. Hartley, *Biochem. J.*, 89, 379 (1963).