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Further Evidence for Formation of 4-Nitrosoquinoline 1-Oxide

In connection with carcinogenic and other biological activities of 4-hydroxyaminoquinoline 1-oxide (4-HAQO),[1] it has been predicted in the previous paper[2] that 4-nitrosoquinoline 1-oxide (4-NOQO) would have been produced as an intermediate in oxidation of 4-HAQO with oxygen in the air, since in a moment right after the addition of base to the alcoholic solution of 4-HAQO the reaction mixture showed deep green color.

4-NOQO is an important compound to elucidate the relationship between carcinogenic activity and chemical structure, because 4-NOQO is the intermediate between 4-nitroquinoline 1-oxide (4-NQO) and 4-HAQO, both of which have strong carcinogenic activities. Many attempts, like reduction of 4-NQO or oxidation of 4-HAQO, have been made to synthesize 4-NOQO by many researchers, but no one has ever succeeded in obtaining the compound. The present communication is concerned with further evidences for formation of 4-NOQO in oxidation of 4-HAQO in an alkaline solution with oxygen in the air.

A solution of 4-HAQO in 0.1% NaOH aq. soln. covered with approximately same amounts of chloroform, was vigorously stirred at room temperature for an hour. The chloroform layer was subjected to chromatography on alumina, and 4-nitroquinoline 1-oxide (I), mp 153° (3% yield), 4,4′-azoquinoline 1,1′-dioxide (II), mp 260° (1.3% yield), Mass Spectrum *m/e*: 316 (M^+), and an unknown compound (III),[3] mp >360° (20% yield), $C_{18}H_{10}O_3N_4$. UV

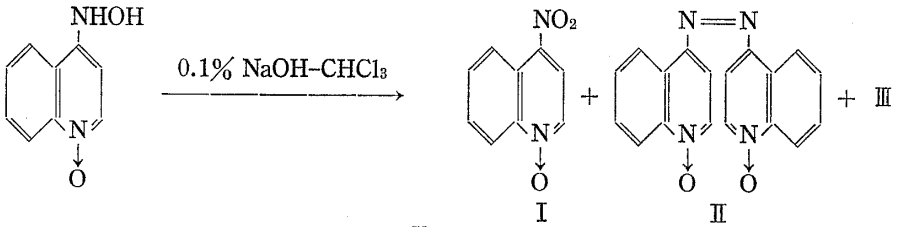

Chart 1

1) *a*) H. Endo and F. Kume, *Naturwissenchaften*, **50**, 525 (1963); *Gann*, **54**, 443 (1963); *b*) Y. Shirasu and A. Ohta, *ibid.*, **54**, 221 (1963); *c*) C. Nagata, N. Kataoka, A. Imamura, Y. Kawazoe and G. Chihara, *ibid.*, **57**, 323 (1966); *d*) Y. Kawazoe and M. Tachibana, *Chem. Pharm. Bull.* (Tokyo), **15**, 1 (1967); *e*) N. Kataoka, S. Shibata, A. Imamura, Y. Kawazoe, G. Chihara and C. Nagata, *ibid.*, **15**, 220 (1967); *f*) Y. Kawazoe, M. Tachibana, A. Aoki and W. Nakahara, *Biochem. Pharmacol.*, **16**, 631 (1967); *g*) M. Hozumi, S. Inuzuka, and T. Sugimura, *Cancer Res.*, **27**, 1378 (1967); *h*) T. Okano and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **16**, 1411 (1968).

2) T. Kosuge and M. Yokota, *Yakugaku Zasshi*, **85**, 69 (1965).

3) We have isolated the same compound as III in the previous work (Ref. 2). And the structure have been proposed as 4,4′-azoxyquinoline 1,1′-dioxide ($C_{18}H_{12}O_3N_4$), since it was identified with one of the reaction products of 4-HAQO and Fehling's solution (E. Ochiai, A. Ohta and H. Nomura, *Chem. Pharm. Bull.* (Tokyo), **5**, 310 (1957).

$\lambda_{max}^{CHCl_3}$ mμ (ε): 270 (67100), 342 (24500), 410 (28300) were obtained. The structure of III will be discussed in the following paper.

Addition of an oxygen donar such as *p*-nitrobenzoic acid, 3,5-dinitrobenzoic acid, or 4-hydroxyquinoline 1-oxide to the reaction mixture resulted in apparent increases of the yield of 4-NQO, while the formation of 4-NQO was completely inhibited by addition of ascorbic acid or hydroxylamine (Table I, and II).

Table I. Results of Addition of Oxygen Donars

Oxygen donar	Yield of 4-NQO (%)	Oxygen donar	Yield of 4-NQO (%)
None	2.7	3,5-Dinitrobenzoic acid	5.2
p-Nitrobenzoic acid	8.9	4-Hydroxyquinoline 1-oxide	8.9

Table II. Results of Addition of Inhibitors

Inhibitor	Mole ratio for 4-HAQO	Yield (%)		
		4-NQO	Azo-comp.	III
None	none	2.7	1.3	20.0
Ascorbic acid	1	0.2	0.3	8.9
Ascorbic acid	2	0	0	0.3
Hydroxylamine	2	0	0.5	2.8

It seemed a reasonable explanation for the results that the addition of an oxygen donar promoted formation of free radical of 4-HAQO and further oxidation, while the addition of ascorbic acid would inhibit formation of the radical as already reported by the research groups[1c] of National Cancer center of Japan. And hydroxylamine would react with an intermediate, probably 4-nitrosoquinoline 1-oxide, to produce diazonium compound, resulting in preventing further proceeding of the reaction to produce 4-NQO.

To confirm the assumption of diazonium compound formation, the reaction was carried out in the presence of both hydroxylamine and 2-naphthol to give deep violet needles (IV), mp 234°. UV λ_{max}^{MeOH} mμ (ε): 217 (61500), 268 (15100), 515 (24400) in 1% yield. IV was proved to be identical with an authentic sample[4] of 4-(2-hydroxynaphthylazo)quinoline 1-oxide prepared by a diazo-coupling reaction of 4-aminoquinoline 1-oxide and 2-naphthol.

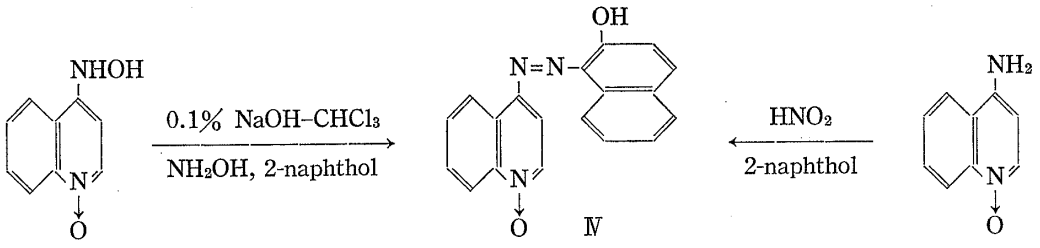

Chart 2

The isolation of IV indicates that the diazonium compound is produced as the intermediate, namely it is a fixed evidence for the formation of 4-NOQO. Besides, the isolation of 4-NQO is also a further evidence for the formation of 4-NOQO, since any other intermediate except 4-NOQO could not be thought from 4-HAQO to 4-NQO. From the both evidences, it is

4) E. Ochiai and T. Naito, *Yakugaku Zasshi*, **64**, 206 (1944).

confirmed that 4-NOQO was formed by oxidation of 4-HAQO with oxygen in basic solution. But it would be probably unable to isolate 4-NOQO as a species stable enough for application to biological tests.

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On the Structure of Glycoside G and K of Bei-Wujiapi

As we reported in the previous papers,[1,2] *n*-BuOH soluble fraction of MeOH extracts of Chinese crude drug, Bei-Wujiapi (cortex of *Periploca sepium* BGE. (Asclepiadaceae)), was revealed to contain many glycosidic substances (A—N) by TLC.[3]

The mixture of glycosides was repeatedly purified by column chromatography affording three crystalline glycosides, tentatively named glycoside G (0.02% from dried material), glycoside H_1 (0.07%) and glycoside K (0.005%).

Glycoside G (I), $C_{36}H_{56}O_{13}$, mp 232—233°, colorless needles from AcOEt saturated with H_2O, $[\alpha]_D^{19}$ +30.2° (c=0.99, EtOH), infrared (IR) ν_{max}^{KBr} cm^{-1}: 3400, 1750, was acetylated with acetic anhydride and pyridine to give a tetraacetate, $C_{44}H_{64}O_{17}$, mp 198°, colorless needles from EtOH–*n*-hexane, $[\alpha]_D^{19}$ +17.8° (c=0.34, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3500, 1750 (broad), 1235. Acid hydrolysis of I with both Kiliani mixture[4] and 0.05N H_2SO_4, yielded periplogenin,[5] D-cymarose, D-glucose, and periplobiose.[6] Enzymatic hydrolysis of I with taka-diastase-A gave D-glucose and product-GE (II), $C_{30}H_{46}O_8$, mp 146°/208° (double melting point), colorless needles from dil. EtOH, $[\alpha]_D^{19}$ +26.41° (c=0.92, 95% EtOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1750 which was identified as periplocymarin[5b] (III) by the mixed fusion and the comparison of TLC and IR spectrum with the authentic sample which was given us by Prof. T. Reichstein. The direct comparison of I with periplocin[6] (IV) has not yet done, but above mentioned characters of I suggest that I must be identical with periplocin. The physical constants of I, II and III, IV are comparatively summarized in Table I.

The second crystalline glycoside-K (V), $C_{40}H_{66}O_{16}$, mp 240—241°, colorless needles from MeOH–AcOEt saturated with H_2O, $[\alpha]_D^{20}$ −27.58° (c=1.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, was methylated by Hakomori's method[7] to yield nona-O-methyl glycoside K (VI), $C_{49}H_{84}O_{16}$,

1) S. Sakuma, S. Kawanishi, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 326 (1968).
2) J. Shoji, S. Kawanishi, S. Sakuma, H. Okino, and M. Sano, *Chem. Pharm. Bull.* (Tokyo), **16**, 2308 (1968).
3) T. Kawasaki, and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), **11**, 1546 (1963).
4) H. Kiliani, *Chem. Ber.*, **63**, 2866 (1930).
5) *a*) E. Ruppol and I. Irukovic, *J. Pharm. Belg.*, **10**, 221 (1955), *b*) P. Brauchli, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **44**, 904 (1961).
6) A. Stoll and J. Renz, *Helv. Chim. Acta*, **22**, 1193 (1939).