added to an ice-cooled solution of IV (50 mg) in 80% aqueous pyridine (3.5 ml) containing 1% triethylamine (0.56 ml). The solution was stirred at 4° for 40 hr and then the solution was lyophilized. The residue was dissolved in H_2O (200 ml), which was applied to a column of CM-cellulose (1.5×8 cm). It was first eluted with H_2O (400 ml) and then the following ammonium acetate buffers (pH 6.9): 0.01 M (500 ml) and 0.02 M (750 ml). Individual fractions (10 ml each) were collected and absorbancy at 280 mμ was determined. The desired fraction in the 0.01 M eluates was collected and the solvent was evaporated *in vacuo*. The residue was lyophilized to constant weight; yield 45 mg (58%), $[\alpha]_D^{22}$ −37.9° (c=0.3, 30% AcOH); Rf_1 0.57, Rf_2 0.66; amino acid ratios in an acid hydrolysate $Ser_{2.01}Tyr_{1.06}Met_{1.06}Glu_{0.97}His_{1.07}Phe_{1.00}Arg_{1.05}Gly_{1.03}Lys_{1.03}Pro_{1.00}$-$Val_{0.91}$ (average recovery 87%). *Anal.* Calcd. for $C_{78}H_{109}O_{20}N_{21}S\cdot CH_3COOH\cdot 10H_2O$: C, 49.7; H, 6.9; N, 15.2. Found: C, 49.7; H, 6.2; N, 14.8.

N$^{\alpha}$-Acetyl-L-seryl-L-tyrosyl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-L-lysyl-L-prolyl-L-valine Amide Diacetate Tridecahydrate (II)——To a solution of V (45 mg) in H_2O (5 ml), 80% hydrazine hydrate (0.18 ml) and thioglycolic acid (0.02 ml) were added and the solution, after adjusting the pH to 6 with AcOH, was incubated at 100° for 3 hr. The solvent was lyophilized and the residue was applied to a column of CM-cellulose (1.5×16 cm), which was first eluted with H_2O (390 ml) and then the following ammonium acetate buffers; 0.01 M (650 ml), 0.025 M (600 ml) and 0.05 M (550 ml). Individual fractions (15 ml each) were collected and measurement of absorbancy at 280 mμ served to locate the desired fraction in 0.025 M eluate, which was pooled and the solvent was evaporated. The residue was lyophilized to constant weight; yield 28 mg (61%), $[\alpha]_D^{29}$ −23.3° (c=0.3, 10% AcOH), Rf_1 0.39, Rf_2 0.49; amino acid ratios in an acid hydrolysate $Ser_{2.10}Tyr_{1.10}Met_{1.08}Glu_{1.10}His_{1.03}Phe_{1.00}Arg_{1.04}Gly_{1.06}Lys_{1.01}Pro_{0.97}$-$Val_{1.00}$ (average recovery 97%). *Anal.* Calcd. for $C_{77}H_{109}O_{19}N_{21}S\cdot 2CH_3COOH\cdot 13H_2O$: C, 48.2; H, 6.3; N, 14.6. Found: C, 48.2; H, 6.3; N, 14.6.

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Syntheses and Properties of Several 4-Alkyl- or Arylsulfonylquinoline 1-Oxides

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It is now well known that 4-nitroquinoline 1-oxide (4-NQO) is one of the most potent carcinogenic and mutagenic agents.[2-5] In 1955, the mutagenic mechanism of this compound was proposed to involve a nucleophilic displacement *in vivo* of the nitro group by sulfhydril group of cystein residue of celluler protein. An alternative could not be excluded that the nitrous acid liberated in the displacement reaction process may play a substantial role in carcinogenesis or mutagenesis of the compound.[6]

1) Location: *a*) *Tsukiji 5-Chome, Chuo-ku, Tokyo*; *b*) *Sapporo, Hokkaido.*
2) W. Nakahara, F. Fukuoka, and T. Sugimura, *Gann*, **48**, 129 (1957).
3) T. Okabayashi, *Hakkokogaku Zasshi*, **33**, 513 (1955).
4) H. Endo, *Gann*, **49**, 151 (1958).
5) W. Nakahara and F. Fukuoka, *Gann*, **50**, 1 (1959).
6) An experimental evidences was recently provided by Okabayashi for the facts that 4-NQO was reductively metabolized to 4-hydroxyamino derivative (4-HAQO), the later compound being proved to be also carcinogen[7] and mutagen.[8]
7) Y. Shirasu and A. Ohta, *Gann*, **54**, 221 (1963).
8) T. Okabayashi and A. Yoshimoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 257 (1964).

In the course of conducting studies of purines and their nucleosides, methylsulfonyl derivatives of purines were found to be easily attacked by nucleophilic reagents. Considering the similarity between nitro and sulfonyl groups, in the reactivity towards nucleophiles, an attempt was made to synthesize and to test the biological activities of these derivatives in order to clarify the structure–biological activity relationships, which is presented in this paper.

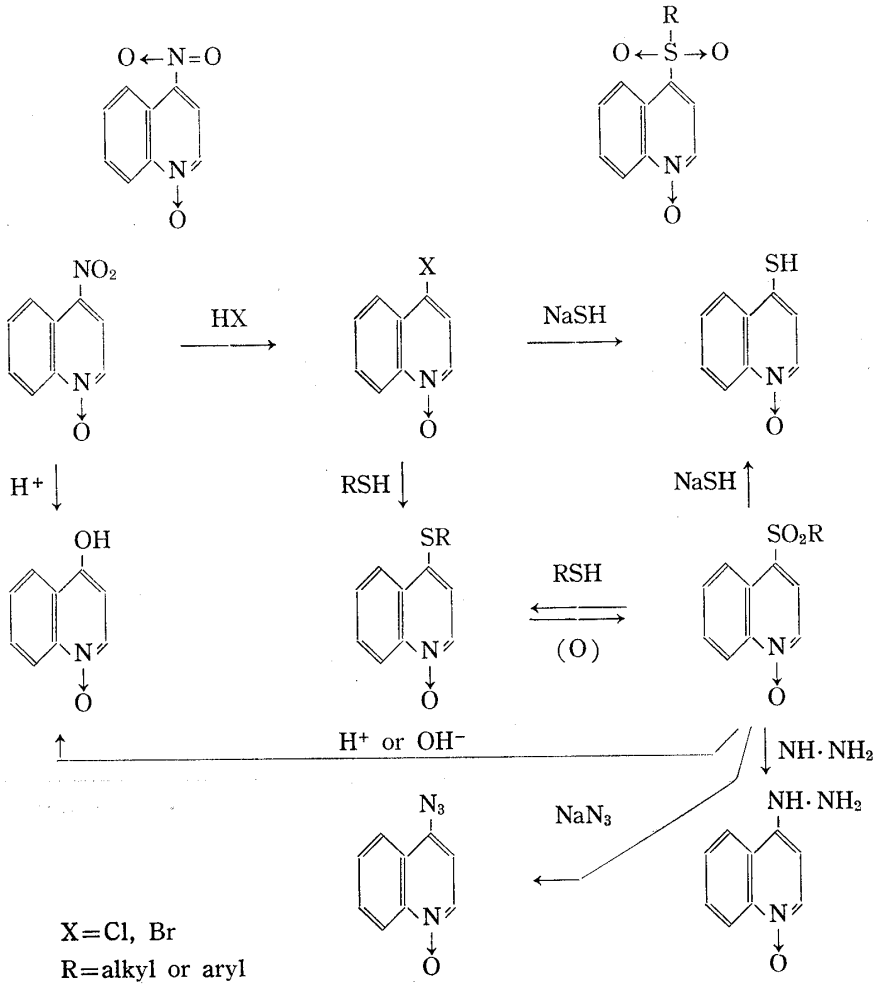


Chart 1

Results and Discussion

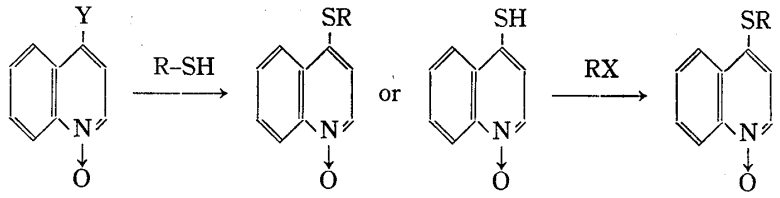
Since direct replacement of the nitro group of 4–NQO (I) with sodium mercaptide gave unsatisfactory results, 4–chloro– or 4–bromoquinoline 1–oxide (IIa), (IIb) respectively, was used as the starting material. Reaction of these halogeno compounds with sodium alkyl– or arylmercaptides in anhydrous methanol afforded easily 4–alkyl– or arylmercaptoquinoline 1–oxides (IV's). Alternatively, (IIa) was converted to 4–mercaptoquinoline 1–oxide (III) by the reaction of sodium hydrogen sulfide in refluxing methanol, followed by treating with alkyl– or aryl halide to give IV's. Phenyl or cresyl derivatives could not be obtained by this method.

These alkyl– or arylmercaptoquinoline 1–oxides were oxidized with hydrogen peroxide in acetic acid or with chlorine gas in methanol to afford sulfonyl derivatives (V's). The structures of these derivatives were established by elemental analytical results and infrared (IR) absorptions, 1160 and 1370 cm^{-1} which are due to $-SO_2-$ streching vibration. Melting points

9) M. Ikehara, A. Yamazaki, and S. Fujieda, *Chem. Pharm. Bull.* (Tokyo), **10**, 1075 (1962).

and natures of 4–alkyl– or arylmercaptoquinoline 1–oxides were summarized in Table I and analytical results of sulfonyl derivatives were summarized in Table II.

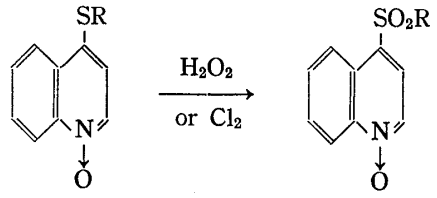
Table I.



Y	R	Yield (%)	X	R	Yield (%)	mp (°C)
Cl	CH_3	55	SO_4	$(CH_3)_2$	77	oil
Br	CH_3	58	I	CH_3	75	oil
Cl	C_2H_5	60	SO_4	$(C_2H_5)_2$	75	oil
Br	C_2H_5	63	I	C_2H_5	78	oil[a]
Cl	$CH(CH_3)_2$	35	Cl	$CH(CH_3)_2$	28	52— 53
Cl	CH_2CH_2OH	35	Cl	CH_2CH_2OH	28	127—129
Cl	$C_6H_5CH_2$	79	Cl	$C_6H_5CH_2$	83	161—162
Cl	C_6H_5	82	—	—	—	98—101
Cl	$NO_2C_6H_4$	89	Cl	$NO_2C_6H_4$	90	121—123

a) Solidified at 0°.

Table II.



R	Yield (%) (A)	Yield (%) (B)	mp (°C)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
CH_3	88	90	210—212	53.77	4.05	6.33	53.76	4.05	6.27
C_2H_5	58	65	128—130	55.69	4.64	5.90	55.99	4.65	5.95
$CH(CH_3)_2$	55	—	174—176	57.60	4.80	5.76	57.81	4.78	5.74
CH_2CH_2OH	38	—	177—178	64.55	4.34	4.68	64.26	4.31	4.63
$C_6H_5CH_2$	80	89	215—216	52.59	4.34	5.57	52.28	4.28	5.74
C_6H_5	85	—	143—144	64.44	3.98	4.87	63.80	4.29	4.99
$NO_2C_6H_4$	90	94	195—196	57.82	3.04	8.48	58.10	3.24	8.79

Refluxing 4–methylsulfonylquinoline 1–oxide (V, R=CH_3) in 40% sulfuric acid gave 4–hydroxyquinoline 1–oxide.[10] The reaction of (V, R=CH_3) with 1 N sodium hydroxide also gave 4–hydroxyquinoline 1–oxide as the main product together with some by–products. Refluxing (V, R=CH_3) in ethanol with sodium hydrogen sulfide or sodium mercaptides gave (III) or (IV's). The reaction of (V, R=CH_3) with hydrazine hydrate in ethanol gave 4–hydrazinoquinoline 1–oxide[11] and the reaction with sodium azide in refluxing acetonitrile converted (V) to 4–azidoquinoline 1–oxide.[11] These reaction products were identified with those obtained directly from 4–chloroquinoline 1–oxide by appropriate procedures.

10) T. Okamoto, *Yakugaku Zasshi*, 71, 299 (1951).
11) T. Itai and S. Kamiya, *Chem. Pharm. Bull.* (Tokyo), 9, 87 (1961); S. Kamiya, *ibid.*, 10, 471 (1962).

The 4–alkyl– or arylsulfonylquinoline 1–oxides prepared present study were tested for carcinogenecity by Dr. W. Nakahara and his co–workers.[12]

The propylene glycol solution (5 mg/ml) of the compound tested subcutaneously injected into the left groin of the mouse in doses of 0.1 ml, the injection being repeated at the same site six times at intervals of days.

But, no tumor induction was observed with any compounds tested by the end of a 250 days period after first injection.

This Nakahara's data may strongly suggest that the nucleophilic replacement reactivity at the 4–position of quinoline 1–oxide ring may not play a substantial role in carcinogenecity.

Antitumor activity of these derivatives are now under investigation.

Experimental

4-Mercaptoquinoline 1-Oxide (III)——Into a solution of anhydrous methanol (150 ml) containing sodium metal (2 g), dry hydrogen sulfide was bubbled for 3 hr. Until the pH of the reaction mixture was examined by bromothymol–blue paper (blue). 4–Chloroquinoline 1–oxide (5 g) was added and resulting mixture was refluxed for 6 hr. Precipitated sodium chloride was filtered off and the filtrate was evaporated *in vacuo*. The residue was taken up in a small amount of water and acidified with acetic acid to pH 2—3. Precipitated material, mp 136—140°, was recrystallized from boiling methanol. Orange–yellow needles, mp 140—141°, was obtained. 3.7 g (75%). UV absorption: λ_{max}^{EtOH} 353 mμ. λ_{min}^{EtOH} 302 mμ. These properties were identical with those reported by Itai[13] and Suzuki.[14]

4-Alkyl- or Arylmercaptoquinoline 1-Oxide (General Procedure) Method A——Alkyl– or arylmercaptan was converted to its sodium salt by the addition of equimolar amount of sodium ethoxide in ethanol. A solution of 4–chloro or 4–bromoquinoline 1–oxide (1 equivalent) dissolved in anhydrous ethanol was added dropwise with stirring during 15 min. The reaction mixture was refluxed for 1.5—2 hr. After cooling, precipitated sodium chloride was removed by filtration and the filtrate was concentrated *in vacuo*. Water was added to the syrup and the mixture was extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol, ethanol or acetone. Yield and properties were listed in Table I.

When only an oily material was obtained as reaction product, it was used directly in the next step without further purification.

Method B: 4–Mercaptoquinoline 1–oxide (III) (15 mmole) was added in 30% sodium hydroxide solution (60 ml) and alkylated either with dialkyl sulfate or alkyl or aryl halide (only *p*–nitrochlorobenzene) (1.1 equivalent). The reaction was performed at room temperature under vigorous stirring for 30 min. Alkyl– or arylmercaptoquinoline 1–oxide was extracted with chloroform and treated by the same procedure as described in method A. Chlorobenzene was not reactive enough for the method B. Isopropyl chloride reacted with (III), when heated at 100° for 1 hr in a seald tube. The yield and properties of the product were summarized in Table I.

4-Alkyl- or Arylsulfonylquinoline 1-Oxide (General Procedure)——Method A: Alkyl– or arylmercaptoquinoline 1–oxide (20 mmole) was dissolved in glacial acetic acid (6 ml) and 30% hydrogen peroxide was added (2–equivalent). After 1 hr at 65°, 30% hydrogen peroxide (1 equivalent) was added and heating was continued for 2 hr. The reaction mixture was concentrated *in vacuo* and a saturated sodium hydrogen carbonate was added to the residue. The mixture was extracted by chloroform and extract was dried over anhydrous magnesium sulfate. Solvent was removed under reduced pressure and residual mass was recrystallized from methanol or acetone. The yield ranged between 75—85%. Physical properties and analytical results were listed in Table II.

Method B: Alkyl– or arylmercaptoquinoline 1–oxide (10 mmole) was dissolved in 70% methanol (40 ml) and chlorine gas was bubbled through the solution below 10° for 30 min. The reaction mixture was heated at 50—60° for 1 hr on a water bath. Methanol was removed by vacumm distilation and the resulting residue was recrystallized from methanol or acetone. Physical properties are listed in Table II. IR absorption band: γ_{max}^{KBr} 1160, 1370 cm^{-1}(–SO_2–); 1295—1300 cm^{-1} (heterocyclic N–oxide)

Reaction of 4-Methylsulfonylquinoline 1-Oxide with 40% Sulfuric Acid——4–Methylsulfonylquinoline 1–oxide (190 mg) was dissolved in 40% sulfuric acid (20 ml) and heated at 100° for 1 hr. After cooling, reaction mixture was neutralized with sodium carbonate solution. A crystalline material was precipitated as light yellow needles at pH 3—4. Collection and recrystallization from methanol gave 4–hydroxyquino-

12) W. Nakahara and K. Aoki, private communication.
13) T. Itai, *Yakugaku Zasshi*, **69**, 542 (1949).
14) Y. Suzuki, *Yakugaku Zasshi*, **81**, 5997 (1959).

line 1-oxide, mp 238—240°. This sample was identified with an authentic specimen described by Okamoto.[10]

Reaction of 4-Methylsulfonylquinoline 1-Oxide with 1N Sodium Hydroxide——4-Methylsulfonylquinoline 1-oxide (190 mg) was dissolved in N sodium hydroxide (20 ml) and gently warmed on a water bath. The reaction mixture began to be colored and muddy precipitate appeared. After heating for 1 hr at 70°, the precipitate was dissolved in water and examined by paper chromatography (solvent: water pH 10). A spot (Rf=0.86) corresponding to 4-hydroxyquinoline 1-oxide was observed as the main product, accompanied with two (Rf=0.56, 0.42) minor spots.

Reaction of 4-Methylsulfonylquinoline 1-Oxide with Sodium Hydrogen Sulfide——Into a solution of sodium hydrogen sulfide, an ethanol solution of 4-methylsulfonylquinoline 1-oxide (45 mg) was added. The resulting mixture was refluxed for 3 hr and evaporated to dryness. The red colored residue was taken up in small amount of water and acidified with acetic acid to pH 2—3. Then, precipitated material was collected and recrystallized from methanol. Orange-yellow needles. mp 140—141°. This sample was identified by mixed melting point test and IR absorption measurement with authentic specimen.

Reaction of 4-Alkyl- or Arylsulfonylquinoline 1-Oxide with Alkyl- or Arylmercaptan——Into an ethanol solution of sodium alkyl- or arylmercaptide, an ethanol solution of 4-alkyl- or arylsulfonylquinoline 1-oxide (1 equivalent) was combined. The solution was refluxed gently for 1 hr on an oil bath. Precipitated inorganic salt was removed by filtration, water was added and the product extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness. The product was washed thoroughly with ether and recrystallized from methanol or acetone. This sample was identified with the specimen obtained from 4-chloroquinoline 1-oxide by mixed melting point test and or IR absorption measurement. γ_{max}^{KBr} 700—800 cm^{-1}, 1350 cm^{-1}(-SR), 1295—1300 cm^{-1} (heteroaromatic N-oxide).

Reaction of 4-Methylsulfonylquinoline 1-Oxide with Hydrazine Hydrate——To a solution of 4-methylsulfonylquinoline 1-oxide (190 mg) dissolved in 5 ml of ethanol, 5 ml of 80% hydrazine hydrate was added and the mixture was gently refluxed for 1 hr. Yellowish green needles were precipitated, filtered, and washed with ether and recrystallized from boiling ethanol. Yield 128 mg. mp 168—170° (decomp.). This sample was identical with authentic specimen of 4-hydrazinoquinoline 1-oxide described by Itai and Kamiya.[11]

Reaction of 4-Methylsulfonylquinoline 1-Oxide with Sodium Azide——A mixture of 4 methylsulfonyl quinoline 1-oxide (190 mg), sodium azide (198mg) and 10 ml of acetonitrile was refluxed for 6 hr. After cooling, insoluble salt was filtered off and the filtrate and washings were combined and solvent was removed under reduced pressure. The brown viscous residue was taken up in chloroform and dried over anhydrous magnesium sulfate and solvent was evaporated off. Recrystallization from acetone gave pale brown crystal. Yield 98 mg. mp 141—143° (decomp.). This sample was identical with the specimen of 4-azidoquinoline 1-oxide described by Itai and Kamiya.[11]

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Chemistry of 3-Phenylcholestan-3-ols[1]

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In the preceding paper one of the authors reported that the structure of the Zimmermann complex derived from cholestan-3-one was elucidated to be 2α-(2,4-dinitrophenyl)cholestan-

1) This paper constitutes Part XVII of the series entitled "Analytical Chemical Studies on Steroids"; Part XVI: *J. Chromatog.*, **31**, 535 (1967).
2) Location: *Kita-4-bancho, Sendai.*