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Synthesis and Characterization of Chinoform Sulfate

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Since its potential sterilizing activity was discovered by David in 1933,[2] chinoform (5-chloro-7-iodo-8-quinolinol) (I) has been widely used for the treatment of intestinal amebiasis. Recently, a possible relationship between the so-called SMON (Subacute myelo-optico neuropathy) disease and chinoform has been pointed out,[3] and examinations on the toxicity of chinoform are being extensively carried out by a number of investigators. In 1953, Haskins, *et al.*[4] reported the presence of sulfate and glucuronide of chinoform in the butanol extract obtained from the urine of chinoform-fed rabbits. More recently, Matsunaga and Tamura[5] reported an authentic synthesis of chinoform glucuronide (5-chloro-7-iodo-8-quinolyl-β-D-glucopyranosiduronic acid).

In this paper, we report the synthesis and properties of chinoform sulfate (5-chloro-7-iodo-8-quinolyl sulfate) (II), and identification of II isolated from the urine of chinoform-fed rabbits.

Result and Discussion

Sulfation of chinoform was unsuccessful by the use of chlorosulfonic acid in pyridine which is one of the usual sulfation procedures, due to its remarkable side reaction. Triethylamine sulfur trioxide complex in dimethylformamide, which is known as an elegant reagent for the sulfation of carbohydrate compounds,[6] did not react with chinoform. After several trials, sulfation of chinoform was successfully achieved with chlorosulfonic acid in the presence of dimethylaniline in chloroform at room temperature,[7] and crystalline sodium salt of II was obtained in 87.4% yield.

Confirmation of the presence of one molar hydrolyzable sulfate and each proton on carbons in 2, 3, 4, and 6 position of II proved that no change had occurred in the chinoform skeleton during this sulfation. The infrared (IR) spectrum on II reveals two strong absorptions at 1240 (ν SO_2) and 1035 (ν SO) cm^{-1}, characteristic of sulfate groups (Fig. 1-b). A comparison of ultraviolet (UV) spectra of II with that of chinoform shows that the introduction of a sulfate group into chinoform molecule resulted in lowering of the wave-length of its maximum absorption over all the pH range (Fig. 2-b). A similar effect due to sulfation has also been observed on 8-quinolinol.[8] However, in contrast to 8-quinolinol, which acquires fluorescence by sulfation, II was nonfluorescent over all the pH range tested.[9] This contrary phenomenon is probably due to the effect of heavy atoms at C5 and C7 of quinoline skeleton.[10]

1) Location: *9-1 Shirokane 5 chome, Minato-ku, Tokyo, 108, Japan.*
2) N.A. David, H.G. Johnstone, A.C. Reed, and C.D. Leake, *J. Am. Med. Assoc.*, **100**, 1658 (1933).
3) M. Yoshioka and Z. Tamura, *Igaku No Ayumi*, **74**, 320 (1970).
4) W.T. Haskins and G.W. Luttermoser, *J. Pharmacol. Exp. Therap.*, **109**, 201 (1953).
5) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 1056 (1971).
6) R.L. Whistler and W.W. Spencer, "Methods in carbohydrate chemistry," Vol. 4, by R.L. Whistler, Acad. Press, New York and London, 1964, p. 297.
7) When chinoform was reacted with the recommended reagent at higher temperatures, for example 50°, C-sulfonation of both chinoform and dimethylaniline occurred besides sulfation of chinoform.
8) R.W. Hay and J.A.G. Edmonds, *Chem. Commun.*, **969**, (1967).
9) K. Nagasawa and H. Yoshidome, unpublished work.
10) E.J. Bowen and F. Wokes, "Fluorescence of Solutions," Longmans, London, 1953.

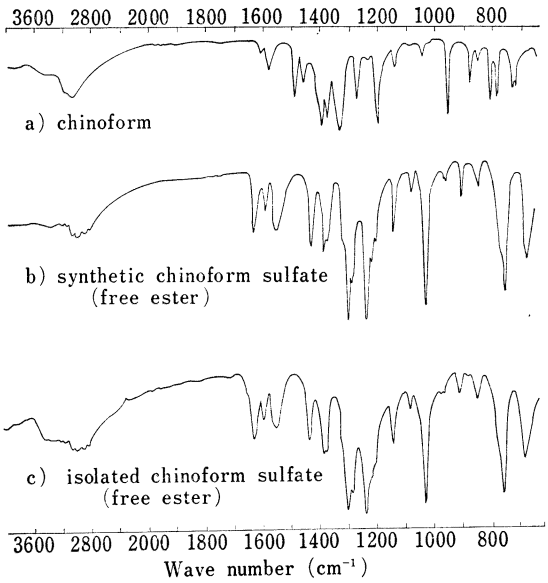

Fig. 1. IR Spectra of Chinoform and Synthetic and Isolated Chinoform Sulfates

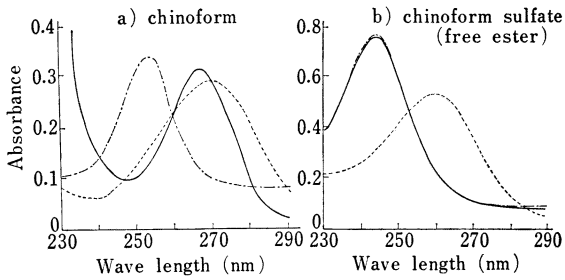

Fig. 2. UV Spectra of Chinoform and Chinoform sulfate

a) ———: 1×10^{-5}M in 10%(v/v) alcoholic 1N NaOH
---------: 1×10^{-5} M in 10% (v/v) alcoholic 1N HCl
—·—·—: 1×10^{-5} M in 10% (v/v) alcoholic water

b) ———: 2×10^{-5}M in 1N NaOH
---------: 2×10^{-5} M in 1N HCl
—·—·—: 2×10^{-5} M in neutral water

It is known that Cu^{2+} enhanced not only the rate of hydrolysis of 8-quinolyl sulfate[8] but also its solvolytic reactivity.[9] The effect of various metal ions on the rate of hydrolysis of II was examined. As shown in Table I, Cu^{2+}, Pd^{2+}, and Hg^{2+} markedly accelerated the rate of hydrolysis of chinoform sulfate in neutral medium at room temperature and the effect of Cu^{2+} was especially notable. In the case of Ca^{2+}, Zn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Sr^{2+}, solution of chinoform sulfate remained unchanged in appearance and inorganic sulfate was also not detected even after 4 hr. In the presence of Fe^{3+}, a trace of greenish precipitate was formed.

II gradually decomposed even in the absence of metal ions; the rate of its decomposition was 0, 5, and 50% after 7, 24, and 100 hr, respectively, in neutral aqueous solution at room temperature, but in 0.5N NaOH no detectable decomposition occurred. Quantitative ex-

Table I. Decomposition of Chinoform Sulfate (Na salt) in the presence of Metal Ions

Metal ion added[a]	Decomposition time(min)	Degree of decomposition (%)	Precipitate formation
Cu^{2+}	0	63.5	yellow precipitate
	5	100.0	
Pb^{2+}[b]	10	38.5	yellowish
	30	79.9	brown precipitate
	60	94.3	
Hg^{2+}	30	27.0	yellow precipitate
	60	47.8	
Fe^{3+}	120	0[c]	a trace amount of greenish precipitate
Cd^{2+}, Zn^{2+}, Al^{3+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Sr^{2+}	240	0	no precipitation

a) Metal chloride was used as a source of metal ion except Hg^{2+} which was used as its acetate.
b) $PbCl_2$ was dissolved in 1 N HCl and the acidic solution was used for this experiment without neutralization.
c) Formation of a trace amount of greenish precipitation was observed, but no inorganic sulfate was detected by Dodgson's turbidimetry.

periment in acidic medium was impossible because of less solubility of II in 0.5N HCl, but it might decompose moderately, for the spectrum of II converted to that of chinoform during measurement of UV spectra in 1N HCl.

It was notable that II decomposed to chinoform and inorganic sulfate in anhydrous hexadetuerodimethyl sulfoxide used as a solvent during the measurement of its proton magnetic resonance (PMR) spectrum. The signal of C6 proton of II decreased as that of C6 proton of chinoform increased and finally the signal of the former disappeared and that of the proton of sulfuric acid appeared. In paper electrophoresis of the solution used for PMR measurement, spots of chinoform and inorganic sulfate were recognized but that of II could not be detected. As shown in Table II, the rate of decomposition of II in anhydrous dimethyl sulfoxide was markedly increased with an increase in temperature. A similar solvolytic desulfation in polar organic solvent has been observed in some steroid alcohol sulfates,[11] carbohydrate sulfate,[12] and 8-quinolyl sulfate.[9]

Table II. Decomposition of Chinoform Sulfate (Na salt) in Dimethyl Sulfoxide

Reaction condition Temperature (C°)	Time (min)	Degree of decomposition (%)
35	90	2.6
	150	6.3
	300	26.8
50	30	10.0
	120	90.9
	180	100.8

Conjugated chinoform fraction was obtained from urine of chinoform-fed rabbit by butanol extraction which was the same procedure used by Haskin, *et al.*[4] The presence of a small spot corresponding to that of the synthetic chinoform sulfate was confirmed by both thin-layer chromatography (TLC) and paper electrophoresis. Substance corresponding to the spot was purified by repeated column chromatography over Avicel and subsequent recrystallization. As shown in Fig. 1, IR spectra of purified substance and of the synthetic sample were identical and, UV spectra at various pHs ($\lambda_{max}^{N\ HCl}$ 260 nm, $\lambda_{max}^{H_2O\ (pH\ 7)}$ 244 nm, $\lambda_{max}^{N\ NaOH}$ 244 nm) agreed with these of the synthetic sample. Mobilities of purified sample on TLC and paper electrophoresis were also identical with that of the synthetic sample.

Experimental

Material——Purified chinoform (mp 181°, sublimed at 170—175°) was obtained by recrystallization of commercially available chinoform. The organic solvents and reagents were all special reagent grade, and used without further purification except chlorosulfonic acid, N,N-dimethylaniline, and $CHCl_3$ which were dried and distilled by the usual procedure.

Analytical Procedures——The total and hydrolyzable sulfur content were determined by the oxygen combustion method and Dodgson's hydrolytic method followed by turbidimetry of inorganic sulfate formed, respectively. Paper electrophoresis was carried out on Toyo Roshi No. 51 paper with a buffer solution (pH 5.8) consisting of pyridine (5 ml), AcOH (1 ml), *n*-BuOH (5 ml), and H_2O (250 ml), at a potential of 23 V/cm, for 40 min. TLC on cellulose powder, Avicel SF was carried out in *n*-BuOH saturated with 1N NH_4OH. The spots were detected under UV ray as dark spots on a light background. PMR spectra were measured at 35° with a Varian T-60 NMR spectrometer operated at 60 MHz in 10—12% solutions of samples in $(CD_3)_2$-SO containing DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) as an internal standard. Chemical

11) S. Burstein and S. Liebermann, *J. Am. Chem. Soc.*, **80**, 5235 (1958).
12) A.I. Usov, K.S. Adamyants, L.T. Miroshnikova, A.A. Shaposhnikova, and N.K. Kochetkov, *Carbohyd. Res.*, **18**, 336 (1971).

shifts were expressed in ppm on δ scale. IR spectra were measured in KBr disc with a JASCO Model IR-S and UV spectra were measured with Hitachi Recording Spectrophotometer EPS-3.

Sodium 5-Chloro-7-iodo-8-quinolylsulfate——A $CHCl_3$ solution (10 ml) of chlorosulfonic acid (5.3 g, 0.05 mole) was added dropwise to a solution of N,N-dimethylaniline (12.1 g, 0.1 mole) in $CHCl_3$ (250 ml) at -2—0°. Recrystallized chinoform (9.15 g, 0.03 mole) was added to this solution and the reaction mixture was stirred for 5 hr at room temperature. After the reaction, the precipitate was collected, washed with dry $CHCl_3$, and dried in air. The dried precipitate was dissolved in warm 1N NaOH to form a homogeneous solution (pH 7—8) which was allowed to stand in the cold to yield pale yellow needles (10.69 g, 87.4%), mp 190—191.5° (decomp.); UV λmax nm (ε); 244 (39000) in H_2O, 260 (26000) in 1N HCl, 244 (38000) in 1N NaOH. *Anal.* Calcd. for $C_9H_4O_4NSClINa$: C, 26.52; H, 0.99; N, 3.44; S, 7.87. Found: C, 26.93; H, 1.25; N, 3.20; S, 7.30 (oxygen combustion method), 7.30 (hydrolytic method).

An aqueous solution of the sodium salt was added to an equivalent amount of 0.1N HCl and kept in the cold to separate thin-brownish needles of free chinoform sulfate. The crystals were collected on a glass filter and washed with a small amount of cold water, then dried over P_2O_5 *in vacuo*. The yield of the free ester, mp 191—193° (decomp.), was almost quantitative.

Decomposition of Chinoform Sulfate in Aqueous Solution——a) With Metal Ions: Sodium chinoform sulfate was dissolved in H_2O to make a 10 mmole solution. A mixture of this solution (0.5 ml) and 0.5M metal chloride (0.5 ml) was reacted at room temperature (20—25°). An aliquot (0.1—0.2 ml) was removed from the reaction mixture at intervals and added to a small amount of Dowex 50 (H^+ form) resin to remove metal ions. The supernatant of this reaction mixture was analyzed for inorganic sulfate.

b) Without Metal Ions: Sodium chinoform sulfate (*ca.* 60 mg) was dissolved in 5 ml of either H_2O, 0.5N NaOH, or 0.5N HCl, and each of the solutions was allowed to stand at room temperature (30°). An aliquot (0.1—0.2 ml) of this solution was removed at intervals and analyzed for inorganic sulfate.

Decomposition of Chinoform Sulfate in Me_2SO——Sodium chinoform sulfate (*ca.* 60 mg) was dissolved in 5 ml of anhyd. Me_2SO to make a 0.15 mmole solution, and the solution was allowed to stand at 35° or 50°. An aliquot of this solution was removed at intervals and analyzed for inorganic sulfate.

Isolation of Chinoform Sulfate from the Urine of Chinoform-fed Rabbit——A suspension of chinoform (4% (w/v), 12.5 ml) in 1% starch solution was administered once daily to a male rabbit (body weight, 2.5 kg) orally, and the urine excreated was collected for 3 days. The urine (425 ml, pH *ca.* 10) was extracted with five 200 ml portions of *n*-BuOH and the combined extract was evaporated to dryness below 40° at reduced pressure. Chromatographical examinations of this dried extract revealed the presence of a small spot whose *Rf* value coincided with that of synthetic chinoform sulfate, together with many large spots of unknown substances. The dried extract was dissolved in a small volume of *n*-BuOH saturated with H_2O and chromatographed over a column (2 × 20 cm) of Avicel using *n*-BuOH saturated with 1N NH_4OH as an eluting medium. The fractions containing the aimed material were combined and concentrated into a small volume at reduced pressure. This concentrate was rechromatographed under the same conditions to give a more purified material (11 mg). This material was chromatographed again over a column (1 × 20 cm) of Avicel using the same solvent to give a fairly purified material. This purified material was dissolved in H_2O and the solution was acidified by the addition of a small amount of Dowex 50 (H^+ form), then filtered. The clear solution which is slightly colored was kept in the cold to separate thin-brownish precipitate (*ca.* 2 mg). The chromatographic and spectrometric analyses were carried out with this purified material.

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