superimposable upon those of natural discretine. Moreover, the spectral data, chromatographic behavior and mp of XXVII were identical with those of (±)-discretine[9] prepared by one of the present authors.

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# Synthesis of 5-Chloro-7-iodo-8-quinolinol Sulfate

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In 1953, Haskins and Luttermoser[2] first suggested the existence of chinoform sulfate and glucuronide in the urine of rabbits administered 5-chloro-7-iodo-8-quinolinol (clioquinol or chinoform) (I), and later Liewendahl, *et al.*[3] demonstrated their existence in human urine after enzymic hydrolysis. However, all these results were obtained without authentic samples. For the ultimate identification of the metabolites and for further studies of the metabolism of I in relation to SMON (subacute myelo-optico-neuropathy),[4] we have tried to synthesize both metabolites. The glucuronide has already been synthesized in our laboratory.[5] The present paper deals with the synthesis of the sulfate (II).

After preliminary experiments for reaction condition, a system of chlorosulfonic acid N,N-dimethylaniline in benzene was finally employed. The reaction proceeded at room temperature. The resulting precipitate was neutralized with an alkaline solution under ice

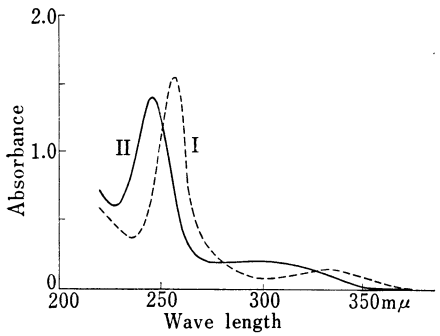


Fig. 1. UV Spectra of Chinoform(I) and Na Salt of Chinoform Sulfate(II)

I: $2.7\times10^{-5}$ M in EtOH
II: $2.6\times10^{-5}$ M in EtOH

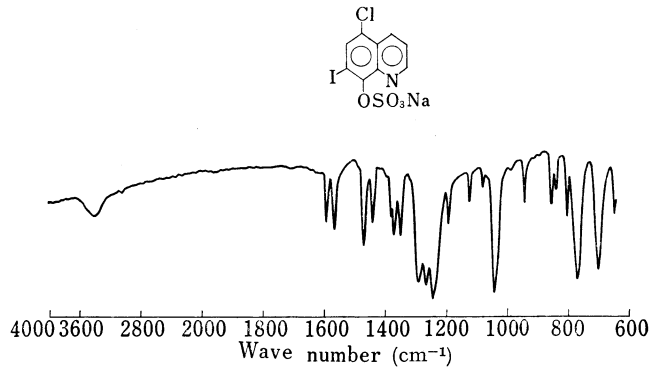


Fig. 2. IR Spectrum of Na Salt of Chinoform Sulfate(II) (KBr Tab.)

1) Location: *Hongo 7-3-1, Bunkyo-ku, Tokyo.*
2) W.T. Haskins and G.W. Luttermoser, *J. Pharmacol. Exptl. Therap.*, **109**, 201 (1953).
3) K. Liewendahl, V. Kivikangas, and B.-A. Lamberg, *Nucl. Med. (Stuttg.)*, **6**, 32 (1967).
4) T. Tsubaki, Y. Toyokura, and H. Tsukakoshi, *J. Japanese Soc. Internal Med.*, **53**, 779 (1964) (in Japanese).
5) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 1056 (1971).

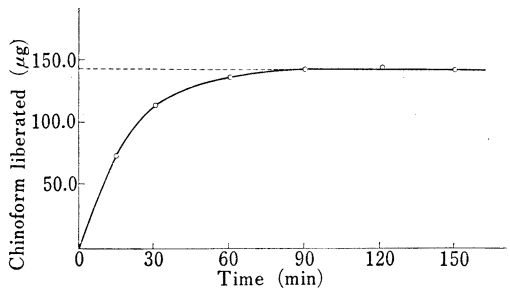


Fig. 3. Acid Hydrolysis of II in 0.1N HCl at 60°
Dotted line shows 100% liberation.

cooling. Although a large amount of precipitate[6] was formed at pH 3.75, the resulting suspension was further neutralized until pH 7.5. Both the crystalline precipitate remaining and the crystals obtained after concentration of the filtrate were elucidated as sodium salt of II. The yield was about 80% through recrystallization. The ultraviolet (UV) and infrared (IR) spectra were shown in Fig. 1 and 2. The evidence of O-sulfate linkage was demonstrated by determining I and sulfuric acid liberated after incubation of the solution with 0.1N hydrochloric acid (Fig. 3), and was also supported by enzymic hydrolysis of Na salt of II with aryl sulfatase.

## Experimental

**Sodium Salt of 5-Chloro-7-iodo-8-quinolinol Sulfate (II)**——Recrystallized I (2.01 g) was dissolved in a mixture of anhydrous benzene (300 ml) and N,N-dimethylaniline (5 ml), and to the mixture 2 ml of chlorosulfonic acid was added dropwise with vigorous stirring in an ice bath. After the addition was completed, the ice bath was removed, and the stirring was continued throughout the reaction for 5 hours at room temperature. At the end of the reaction, most of the pale yellowish brown precipitate produced deposited in the flask. Then the benzene solution was removed by decantation, and the precipitate was treated with saturated solution of sodium bicarbonate (25 ml×2) in an ice bath. The resulting suspension was transferred to a beaker (200 ml) in an ice bath, and neutralized with 1N sodium hydroxide using pH meter. A large amount of precipitate was formed at pH 3.75, and with continuous neutralization considerable amount of the precipitate was redissolved. When the pH of the suspension reached 7.5, neutralization was stopped and the pale brown crystalline precipitate was filtrated. The precipitate was washed with small amount of cold water and about 50 ml of ether (yield, 1.09 g). The filtrate was washed with ether to remove N,N-dimethylaniline and trace amount of I, and concentrated under reduced pressure without heating to get colorless flocculent crystals (yield, 1.16 g). The both crystals with the same physical and chemical characteristics were combined and dissolved in minimal amount of distilled water at room temperature, and the solution was filtered. Recrystallization was carried out similarly by concentrating the filtrate, and resulting bulky crystals were separated. Further crystals were obtained by continuous concentration of the filtrate. (total yield 2.05 g, 77%). TLC: *Rf*, 0.74 on Wakogel UA Plate[7] with *n*-BuOH–PrOH–0.1N $NH_3$ (3: 2: 5 v/v), a violet spot under 254—350 mμ. NMR (DMSO-$d_6$) δ: 7.67 (1H, dd, $J$=8, 4 cps; 3-H), 8.08 (1H, s, 6-H), 8.47 (1H, dd, $J$=8, 2 cps; 4-H), 8.96 (1H, dd, $J$=4, 2 cps; 2-H). *Anal.* Calcd. for $C_6H_4O_4NSClINa$: C, 26.53; H, 0.99; N, 3.44. Found: C, 26.47; H, 0.98; N, 3.53.

**Enzymic Hydrolysis of Na Salt of II**——One ml portions of the substrate solution (containing 330 μg of Na salt of II in one ml of 0.2M acetate buffer, pH 5.0) were incubated with 0.1 ml enzyme (8.0 units aryl sulfatase, E.C. 3.1.6.1, from Limpets, Type III, Sigma) at 37° for 5 hours. An incubation mixture was extracted with benzene, and liberated I in the extract was subsequently adsorbed on a small aluminium column. Strong yellowish green fluorescence appeared suggesting the formation of I-aluminium complex. I in the hydrolysate was also confirmed by gas chromatography.[8]

**Acid Hydrolysis of Na Salt of II**——(1) To a series of glass stoppered centrifuge tubes (10 ml) containing 0.5 ml each of an aqueous solution of Na salt of II (0.38 mg/ml), equal volumes of 0.2N hydrochloric acid were added. Hydrolysis was carried out at 60° at various time intervals (0, 15, 30, 60, 90, 120, and 150 min). Each reaction mixture was extracted with 4 ml of benzene–pyridine (9: 1 v/v). The absorbance of organic

6) It is probable that the precipitate mainly consists of inner salt of II, since by acidifying an aqueous solution of sodium salt of II to pH 3.75, yellow crystals were precipitated, which contained no sodium (mp 138.5—139.5°. *Anal.* Calcd. for free II, $C_9H_5O_4NSCII$: C, 28.04; H, 1.31; N, 3.63. Found: C, 27.94; H, 1.35; N, 3.82). However, the inner salt was spontaneously hydrolyzed in water probably because of its protonated nitrogen. Hence we tried to obtain a stable salt of II.

7) Z. Tamura, C.S. Kim, N. Hosoda, S. Takitani, M. Suzuki, M. Suzuki, and M. Inoue, *Bunseki Kagaku*, **19**, 518 (1970).

8) Presented at the meeting of SMON Research Commission, December 15, 1971.

phase was measured directly at 350 mμ, at which the solvent itself showed negligible absorbance. The time course of the hydrolysis is shown in Fig. 3. The complete hydrolysis was attained in 90 min at 60°, and chinoform liberated was analyzed as 99.6%.

(2) To the solution of Na salt of II (12.4 mg, 0.0304 mmole in 1 ml of water) was added 1 ml of 0.2N HCl, and the mixture was incubated at 60° for 2 hr. The solution was turned yellow and needle crystals appeared. The crystals (9.3 mg, 0.0304 mmole) was identified as I with TLC using authentic chinoform. The supernatant was heated with one ml of 10% $BaCl_2$ solution and immediate precipitation of $BaSO_4$ (7.2 mg, 0.0308 mmole) was observed.