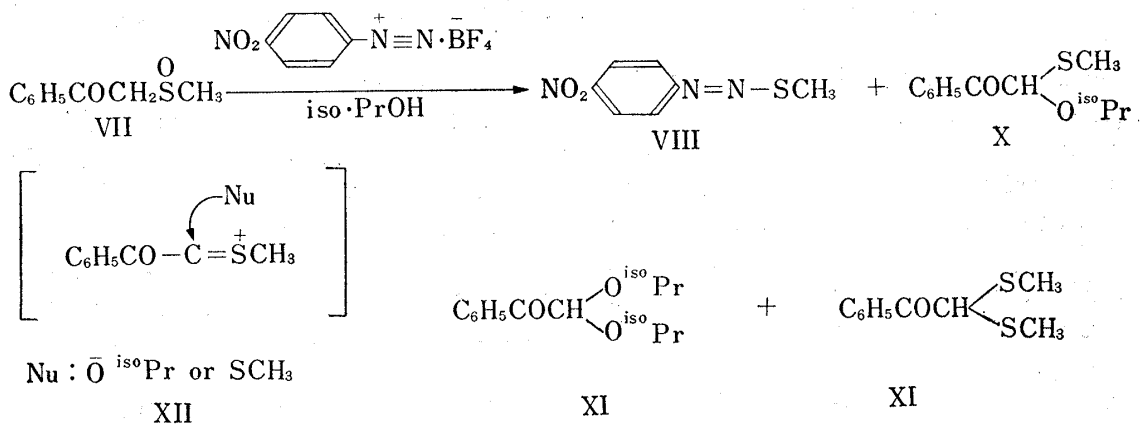

Chart 2

Product X. mp 88°. IR ν_{max}^{Nujol} cm^{-1}: 1675, 1090. UV $\lambda_{max}^{ethanol}$ nm: 246. NMR δ: *ca.* 8.0 (2H, m), *ca.* 7.50 (3H, m), 5.77 (1H, s), 4.12 (1H, sept, J=6 Hz), 2.20 (CH_3S-), 1.22 (3H, d, J=6 Hz), 1.32 (3H, d, J=6 Hz). Mass spectrum: 224 (M^+, $C_{12}H_{16}O_2S$).

Product XI. mp 65° (lit. 66—67°).[9] IR ν_{max}^{Nujol} cm^{-1}: 1675. UV $\lambda_{max}^{ethanol}$ nm: 246. NMR δ: *ca.* 8.0 (2H, m), *ca.* 7.55 (3H, m), 5.30 (1H, s), 2.15 (6H, $CH_3S\times2$).

These products (IX, X and XI) would be formed by an intermediate of Pummerer type such as XII,[10] and product VIII would be originated from the diazonium salt and methylmercaptan from XII.

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A Novel Method for the Determination of Chinoform

Recently, Tamura and his co-workers[1] have shown the presence of 5-chloro-7-iodo-8-quinolinol (chinoform)[2] in the feces and urine of SMON patients. In the course of the investigation on the binding of chemical substances with proteins,[3] we have been interested in the complexation of this quinoline derivative with serum proteins and prompted to establish a sensitive method for its assay. This compound bears a marked structural resembrance to 8-hydroxyquinoline (oxine). The intense fluorescence of metal oxinates has been utilized in their thin-layer chromatography by Takitani and his associates.[4] This paper describes

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2) Clioquinol (BP).
3) T. Kinoshita, F. Iinuma, I. Moriguchi, and A. Tsuji, *Chem. Pharm. Bull.* (Tokyo), **19**, 861 (1971).
4) S. Takitani, Y. Hata, and M. Suzuki, *Japan Analyst*, **18**, 626 (1969).

a new approach to the estimation of chinoform making use of the fluorescence of its aluminum chelate.

The assay procedure is as follows: Twenty–five milliliter of aqueous solution of chinoform (0.04 to 0.4 μg/ml) is extracted with ether (10 ml×5). The extracts are combined and dried by stirring for 20 min in the presence of anhydrous sodium sulfate (5 g). The mixture is then filtered and the dessicant is washed with 20 ml of ether. The combined filtrates and washings are evaporated to dryness under reduced pressure. The residue is dissolved in 4.0 ml of anhydrous pyridine and to the resulting solution is added 20 μl of a 13% solution of aluminum nitrate nonahydrate. The mixture is centrifuged and the supernatant solution is measured fluorometrically. Excitation and emission wavelengths are 365 and 510 nm, respectively.

The fluorescence of the chelate is stable for 3 hr. The excess of the aluminum reagent has no unfavorable effect on the fluorescence. No remarkable change in the fluorescence was observed when the solutions of aluminum nitrate nonahydrate ranging in concentration from 10 to 70% were examined. Besides aluminum nitrate, the nitrates of zinc, magnesium and calcium were tested as reagents for the assay. The magnesium and calcium salts were found to be as effective as the aluminum salt. On the other hand, no interfering effect was observed when each 50 μg of aluminum, ferric, cupric and magnesium ions were added to the aqueous standard solution of chinoform.

The working curve is linear in the range from 0.04 to 0.4 μg/ml of chinoform. Since the sample is concentrated through the evaporation of the ether extracts, the present method is applicable to very dilute solutions. The average recovery of chinoform (3.06 μg/ml) from its aqueous solutions in five determinations was 100.3% and the standard deviation was 2.8%. The recovery was reduced to 95% when benzene or *n*-hexane was employed as solvent for the extraction. The mean recovery of chinoform (3.06 μg/ml) by extraction with ether from human urine in six determinations was 78.9% and the standard deviation was 2.7%. The cause of this low recovery remains uncertain.

The present method is adequate for the estimation of chinoform in aqueous solution and in volatile solvents. For the clinical use, however, further improvements for obtaining better recovery of the sample are desirable. Application of this procedure to the studies on the protein binding of chinoform is in progress combining with the thermodynamic approach and the details will be discussed elsewhere.

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