

b.wt.<sup>-1</sup><sup>12</sup>, some 30% higher than the values recorded in our experiments, with a distribution of 13.1% to the hepatosplanchnic tissue<sup>11</sup>. Thus, simple calculation reveals that liver blood flow should be 3.75 ml · min<sup>-1</sup> 100 g b.wt<sup>-1</sup>. This closely resembles the value of 3.55 ml · min<sup>-1</sup> 100 g b.wt<sup>-1</sup> obtained with urethane in the present study and consequently urethane anaesthesia in rats may produce a liver blood flow which corresponds most closely to that in conscious animals.

However, in contrast to pentobarbital anaesthesia, urethane in rats appears to produce a reduction in cardiac output and liver blood flow as a result of portal vein catheterization. Although cardiac output is reduced, mean arterial pressure remains unchanged, indicating that the animal is in a state

of compensatory shock<sup>13</sup>. The resulting vasoconstriction in the splanchnic vascular bed<sup>13</sup> may explain the greater reduction in liver blood flow than in cardiac output. Liver blood flow fell by 18% whereas cardiac output was decreased by only 13%.

In conclusion, it appears that although urethane anaesthesia may produce a liver blood flow which is similar to that in conscious animals, the trauma of portal vein catheterization may induce circulatory shock. Thus anaesthesia itself, the choice of general anaesthetic and hepatic portal vein catheterization may have profound effects in pharmacokinetic studies involving drugs which are highly cleared by the liver.

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## Chemical and photooxidation of thiothixene (Navane®): Structure of the thiothixene fluorophor<sup>1</sup>

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**Summary.** The spontaneous photooxidation of thiothixene (Navane®) to 2-(N,N-dimethyl-sulfonamido)-9-thioxanthone is described. The corresponding sulfoxide is demonstrated to be the fluorescent species obtained upon permanganate oxidation of thiothixene in the fluorometric assay procedure of Mjörndal and Öreland.

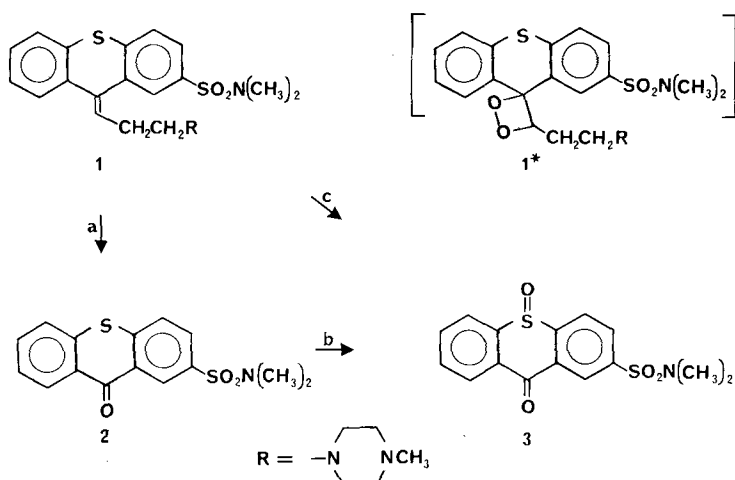
In the course of investigating plasma levels of the thioxanthene neuroleptic thiothixene (**1**) (Navane®), we had occasion to detail 2 previously unreported aspects of the chemistry of the drug which we report herein. These concern the chemical oxidation and the spontaneous photooxidation of thiothixene, both processes which influence the successful assay of low concentrations of the drug.

Although reasonable care was taken to prevent undue exposure of assay solutions to sunlight, we nonetheless noticed on several occasions a photodecomposition occurring in millimolar neutral or dilute acid stock solutions, resulting in the formation of a light yellow precipitate. Isolation of the material by extraction with ethyl acetate, followed by recrystallization from hexane/ethyl acetate afforded a light yellow crystalline substance of m.p. 172–174°C (uncorrected). On the basis of the following spectral data we assigned the thioxanthone structure **2** to the decomposition product. Mass spectrometry demonstrated a molecular ion at *m/e* 319, and major fragments at *m/e* 275 (-NMe<sub>2</sub>), 211 (-SO<sub>2</sub>NMe<sub>2</sub>) and 183 (-CO, -SO<sub>2</sub>NMe<sub>2</sub>). Infrared spectroscopy (20% solution in CHCl<sub>3</sub>) revealed a carbonyl absorption at 1630 cm<sup>-1</sup>, and proton NMR spectrometry (CDCl<sub>3</sub>) showed absorptions at δ (ppm downfield from TMS standard) 2.8 (s, 6H, SO<sub>2</sub>NMe<sub>2</sub>), 7.3–8.1 (m, 5H), 8.6 (m, 1H) and 8.9 (d, 1H). Satisfactory microcom-

bustion analyses were obtained for C<sub>15</sub>H<sub>13</sub>S<sub>2</sub>O<sub>3</sub>N. These data appear to be the same as those reported earlier for this compound<sup>2</sup>.

The formation of **2**, prevented in the absence of direct irradiation or by degassing the solutions with nitrogen, is most likely attributable to the addition of singlet oxygen to the olefin, resulting in an intermediate adduct such as the dioxetane **1**<sup>3,4</sup>, which then collapses to **2**. Once initiated, the reaction would be autocatalytic, the thioxanthone formed being an excellent triplet sensitizer<sup>5</sup>. Alternatively, since phenothiazines are known to form charge transfer complexes with oxygen which then rearrange to hydroperoxides<sup>6</sup>, it is possible that a similar mechanism might be operative in the case of thioxanthenes.

Aside from the possible source of error such an anomalous oxidation could introduce into the assay procedure, we were also concerned about the reaction from another standpoint. We were employing the thiothixene assay procedure of Mjörndal and Öreland<sup>7</sup> which, like phenothiazine assay procedures<sup>8,9</sup> involves permanganate oxidation to generate a fluorescent species, the structure of which has not been reported. In our own hands the permanganate oxidation sometimes resulted in variable calibration curves. We felt that by establishing the structure of the fluorophor we might be able to more carefully control conditions so that the oxidation was always reproducible. We were



The chemical structures of thiothixene (1) and its chemical (3) and photochemical (2) oxidation products. Reaction conditions: a)  $\text{O}_2$ -hv, b) m-Cl-C<sub>6</sub>H<sub>4</sub>CO<sub>3</sub>H, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, c) KMnO<sub>4</sub>, acetate buffer pH 5.5.

reasonably confident that permanganate oxidation of 1 was resulting in formation of the thioxanthone sulfoxide 3. This assumption was based upon known phenothiazine chemistry<sup>9,10</sup> and upon the pH profile for the oxidation reported by Mjörndal and Orelund, where optimal fluorescence was obtained between pH 5 and 8. At high pH side chain oxidation would be incomplete with permanganate, whereas at low pH random overoxidation could occur.

We confirmed this hypothesis by utilizing the thioxanthone 2 as a precursor for the quantitative generation of the sulfoxide 3 (meta-chloroperbenzoic acid, methylene chloride, 0°C). The sulfoxide thus generated demonstrated a fluorescence spectrum (emission, 310 nm excitation) identical to that produced upon permanganate oxidation of 1. We are currently investigating selective methods for the direct generation of 3 from 1 in organic extracts of plasma.

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### Inhibitory effect of tiaramide on ADP-induced aggregation in rabbit platelets<sup>1</sup>

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**Summary.** Tiaramide in  $10^{-4}$  or  $10^{-5}$  M depressed the ADP-induced aggregation of rabbit platelets using the turbidimetric method. In modified Chandler's loop method, tiaramide in the same concentration accelerated the restoration of the time course of disaggregation.

Many non-steroidal anti-inflammatory drugs inhibit the release of platelet constituents normally induced by collagen and thrombin<sup>3,4</sup>, but do not inhibit the primary platelet aggregation induced by ADP. Tiaramide (4-[5-chloro-2-oxo-3-benzothiazolyl-acetyl]-1-piperazine ethanol hydrochloride) was synthesized as a water-soluble analgesic and anti-inflammatory drug<sup>5</sup>. The present paper describes the inhibitory effect of tiaramide on ADP-induced aggregation of rabbit platelets and compares the potency among tiaramide, indomethacin and aspirin, using turbidimetric and modified Chandler's loop method<sup>6</sup>.

**Materials and methods.** Blood from a sodium pentobarbital (30 mg/kg, i.m.) anesthetized rabbit was extracted from

the carotid artery into an injection-syringe containing  $\frac{1}{10}$  vol. of trisodium citrate (3.8% w/v). Platelet-rich plasma (PRP) was prepared by collecting the upper part of the supernatant at  $140 \times g$  after 12 min centrifugation at 4°C. Platelet concentration was adjusted at  $60 \times 10^4/\text{cmm}^3$ . After 20 min incubation with the anti-inflammatory drug, ADP was added to PRP and the aggregation was measured in an Evans aggregometer (37°C). In the loop method, a 25-cm long polyvinyl tube with an inner diameter of 4 mm was used. PRP was mixed individually with the anti-inflammatory drug in the tube rotating at 16 rpm for 20 min on a turntable, with an angle of 23 degrees. After adding ADP, the aggregation and disaggregation was observed for 15 min at 25°C.