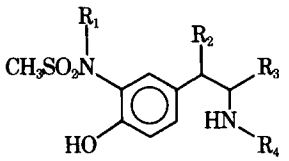
Application of Trihydroxyindole Reaction to Methanesulfonanilides: Fluorometric Analysis for Soterenol and Mesuprine

TERRY T. KENSLER, DANA BROOKE ˣ, and DONNA M. WALKER*

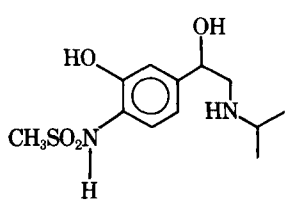
Abstract □ Methanesulfonanilides, like soterenol and mesuprine, which are bioisosteric with adrenergic catecholamines, form fluorescent species when subjected to the trihydroxyindole reaction. Presumably, the fluorescence is due to adrenolutin-like species formed from aminochrome intermediates. Fluorescence was not induced in a relative of soterenol where a methyl group was added to the sulfonamido nitrogen, a fact that suggests the presence of a quinoid intermediate in the reaction scheme of soterenol. A ring isomer of soterenol, where the methanesulfonamido and hydroxyl groups were interchanged, produced only about 5% as much fluorescence response as soterenol. The soterenol counterparts to isoproterenol and isoproterenolsulfonic acid did not produce fluorescence when treated like soterenol. This finding and the fact that response was linear with concentration for soterenol and mesuprine suggest that a fluorometric analysis could be developed for these methanesulfonanilides.

Keyphrases □ Soterenol—fluorometric analysis after trihydroxyindole reaction, pharmaceutical formulations □ Mesuprine—fluorometric analysis after trihydroxyindole reaction, pharmaceutical formulations □ Trihydroxyindole reaction—with methanesulfonanilides, fluorometric analysis of soterenol and mesuprine □ Methanesulfonanilides—trihydroxyindole reaction applied, fluorometric analysis of soterenol and mesuprine □ Adrenergic agents—soterenol and mesuprine, fluorometric analysis, pharmaceutical formulations

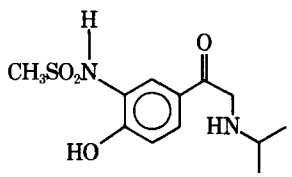
Recently, Prasad *et al.* applied time-honored fluorometric methods to the analysis of epinephrine (1) and isoproterenol (2) in pharmaceuticals. These methods involved iodine oxidation of the catecholamine to the aminochrome, which was subsequently treated to form a fluorescent species. These reports suggested the possibility of fluorometric analyses of certain methanesulfonanilides, which are bioisosteric with catecholamines (3, 4). One such compound, soterenol (I), the methanesulfonamido counterpart of isoproterenol, is well known for its adrenergic activity.



I: $R_1 = H$, $R_2 = OH$, $R_3 = H$, $R_4 = (CH_3)_2CH-$
II: $R_1 = H$, $R_2 = OH$, $R_3 = CH_3$, $R_4 = 4\text{-}(OCH_3)C_6H_4CH_2CH_2-$
III: $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = (CH_3)_2CH-$
IV: $R_1 = H$, $R_2 = SO_3H$, $R_3 = H$, $R_4 = (CH_3)_2CH-$



V



VI

The application of the trihydroxyindole reaction to the analysis of such compounds[1] as I and mesuprine (II) was attempted on the assumption that the sulfonamido group can act like a phenolic hydroxyl group during the formation of aminochromes and, subsequently, fluorescent adrenolutin-like derivatives. Experimental support of this supposition should be of general interest.

As will be seen, methanesulfonanilides like I and II do form fluorescent derivatives. Compounds III–VI were used to examine certain other aspects of the effect of substrate structure on the development of fluorescence.

EXPERIMENTAL

Reagents—Compounds I–VI as the appropriate salts were drawn from laboratory stock. The hydrochloride salts of I, II, V, and VI were prepared and described previously (4). The acetate salt of III is that described in a recent U.S. patent (5).

Compound IV, 1-(4-hydroxy-3-methanesulfonamidophenyl)-2-isopropylaminoethanesulfonic acid, was prepared[2] by heating a solution of equimolar amounts of I as the hydrochloride and sodium bisulfite at pH 6.8 under reflux for 5.5 hr. The solution was cooled and filtered, and the white solid was recrystallized once from 1 *N* HCl and once from water, giving IV, mp 288.5° dec. (corrected). The IR, NMR, UV, elemental, and TLC analyses were consistent with the one compound, IV.

Isoproterenol hydrochloride USP[3] was used as received. All other chemicals were either USP or reagent grade. The water was double distilled.

Buffer—Buffers, 0.1 *M* in citrate, were prepared by dissolving appropriate amounts of sodium citrate and citric acid in water and adjusting the pH with sodium hydroxide when necessary.

Iodine–Potassium Iodide Solution—A 0.001 *M* I_2 in 1.4% KI solution was prepared by diluting 1.0 ml of a 0.1 *M* I_2 (in 0.1 *M* KI) solution to 100 ml with 1.4% KI solution.

Sodium Ascorbate (0.2% in 5 *N* NaOH)—A 5.0-ml aliquot of a freshly prepared 2% solution of ascorbic acid was diluted to 50.0 ml in a volumetric flask with 5 *N* NaOH. This solution was used within 1 hr.

Apparatus—Fluorescence measurements were routinely taken using a ratio fluorometer[4] equipped with No. 405 narrow pass primary filters and a No. 2A-12 sharp cut secondary filter[5]. The fluorescence of each sample was recorded relative to an 1800-ppb quinine sulfate equivalent uranium glass reference bar. Emission spectra were recorded using a spectrophotofluorometer[6].

Procedure—Solutions for analysis, essentially 0.1 *M* in citrate, were prepared by diluting stock solutions of the substrates with citrate buffer. A 1.0-ml portion of the 0.001 *M* I_2 solution was added with agitation to a 10-ml volumetric flask containing 5.0 ml of the buffered sample. After a predetermined time, sodium ascorbate solution was added to volume and the solution was mixed. The rela-

[1] Soterenol and mesuprine have been cited as MJ 1992 and MJ 1987, respectively, in the scientific literature.
[2] D. G. Mikolasek, Mead Johnson and Co., personal communication.
[3] Gane's Chemical Works.
[4] Beckman model 772.
[5] Turner stock Nos. 110-812 and 110-818.
[6] Aminco-Bowman model 8202.

Table I—Fluorometric Measurements with Sulfonanilides and Isoproterenol

Compound	Sample, *M*	Iodine, *M*	pH	Fluoresce	Excitation[a], λ_{max}	Emission[a], λ_{max}
I	1.45×10^{-5}	0.001	5.5	Yes	425	505
II	1.20×10^{-5}	0.001	5.5	Yes	430	510
III	2.90×10^{-4}	0.005	4.0—7.1	No	—	—
IV	8.90×10^{-6}	0.001	5.5	No	—	—
V	6.88×10^{-5}	0.001	5.5	Yes	455	545
VI	1.23×10^{-5}	0.001	5.5	No	—	—
Isoproterenol	3.36×10^{-6}	0.001	4.0	Yes	420	510

[a] Excitation and emission wavelengths are from uncorrected spectra and are only approximate.

tive fluorescence developed was determined after 8–10 min with a reagent blank set at zero.

RESULTS AND DISCUSSION

Compounds I, II, and, to a lesser extent, V can be made to fluoresce by treatment with iodine followed by alkaline ascorbate. The readings are linear with substrate concentration, as illustrated for I and II in Fig. 1. The data of Fig. 1 were based on reaction with iodine at pH 5.5 (I for 15 min, II for 30 min) followed by addition of ascorbate solution with measurement of fluorescence 8–10 min later. The pH and time of the oxidation step were chosen to maximize reproducibility and convenience of sample handling. The time course of fluorescence development for II at three pH values is shown in Fig. 2.

The data of Fig. 1 are intended only to depict linearity of meter response *versus* concentration when a ratio fluorometer is used. When, however, fluorescence spectra are recorded by means of a spectrophotofluorometer for samples containing I or II, treated as described, the responses per mole of substrate, measured at λ_{max} (Table I), are virtually identical. Isoproterenol hydrochloride, oxidized at its optimal pH of 4.0 (2) for 10 min, developed approximately twice the fluorescence per mole. Oxidation of V (10 min at pH 5.5) produced only about one-twentieth of the response recorded for I as described previously[7].

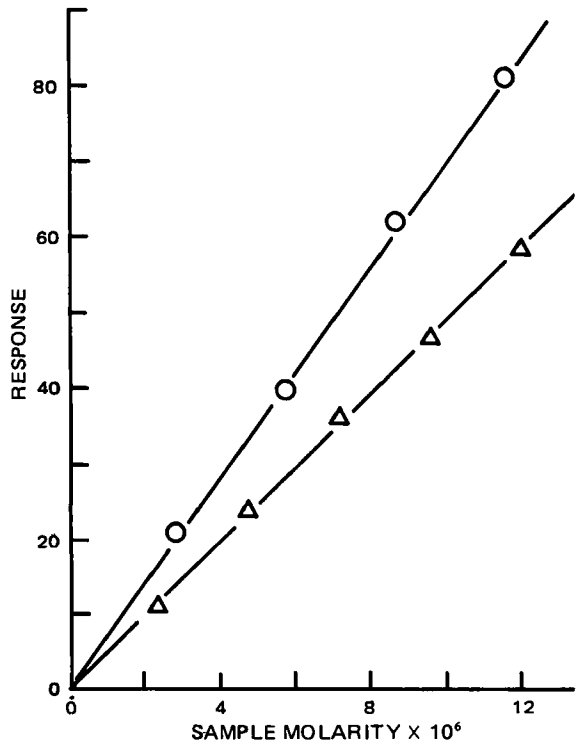


Figure 1—*Ratio of fluorometer response* versus *sample concentration for soterenol* (○) *and mesuprine* (△).

[7] Fluorescence readings were slightly higher if the oxidation was at pH 4.5. At both pH values, the readings were reasonably constant from 3 to 30 min.

These comparisons may aid in judging the reactivities of the substrates tested. However, because numerous factors affect the formation and stability of aminochrome intermediates and the adrenolutin-like fluorophores, the comparisons should be used cautiously. For example, the ultimate concentration of fluorophore can be adversely affected by side reactions such as the iodination of aminochromes. Mattok and Wilson (6, 7) showed that the rate of iodination depends upon iodine and iodide concentrations and is general base (buffer) catalyzed. Laverty and Taylor (8) showed that the oxidation of catecholamines to aminochromes was significantly influenced by buffer composition and pH.

No fluorescence was observed when III was treated under conditions that easily oxidized I. This was true even when the concentration of III (2.9×10^{-4} *M*) was 20 times the highest concentration used for I (1.45×10^{-5} *M*) and the iodine concentration was increased fivefold to 0.005 *M*. In these cases, the oxidation step was attempted for up to 1 hr at pH values ranging from 4.0 to 7.1.

This finding appears to be a parallel to the fact that guaiacolamines do not produce fluorescence under conditions that easily develop fluorescence in catecholamines. For example, normetanephrine produces fluorescence when treated with iodine only under alkaline conditions (8). Metanephrine and normetanephrine are oxidized by metaperiodate under acid conditions where the reaction probably involves loss of the O^3-methyl group (9). This finding indirectly supports the premise that fluorescence development from the methanesulfonanilides requires a quinoid-like intermediate (VII) similar to that proposed (9, 10) for the formation of aminochromes from catecholamines. An extension of this reasoning would support formation of an aminochrome intermediate (VIII) from the methanesulfonanilides. This structure is not unlike the stable *p*-nitrophenylhydrazone (IX) of adrenochrome (11).

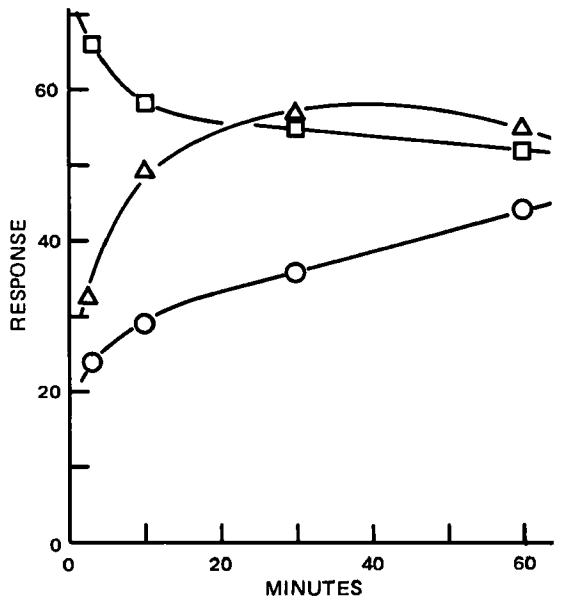
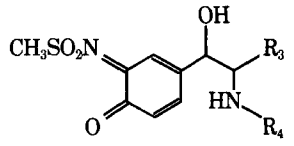
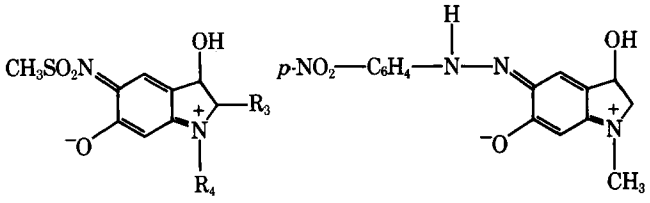


Figure 2—*Effect of time and pH of the oxidation step on the ultimate fluorescence of mesuprine. Buffers used were at pH 4.5* (○), *5.5* (△), *and 6.4* (□). *Mesuprine concentration was 1.2 × 10^{-5}* M.



VII



VIII IX

That V, an isomer of I differing only in the positions of the ring substituents, produced only about one-twentieth the fluorescence of I was somewhat surprising. No explanation is offered, but it is interesting to note that compounds in this series (*i.e.*, those with the methanesulfonamido group *para* to the ethanolamine side chain) also have considerably lower adrenergic potency (4).

Prasad *et al.* (2) showed that isoproterenol and isoproterenolsulfonic acid do not interfere with their fluorometric method. Similarly, the ketone VI and the sulfonic acid IV do not form fluorescent species under the conditions used to analyze I. These final tests were designed only to demonstrate the lack of interference from IV and VI and do not preclude the possibility that these compounds might form fluorescent derivatives under different conditions.

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* Present address: Armak Co., Chicago, Ill.

‡ To whom inquiries should be directed.

Thiocardenolides I: Synthesis and Biological Actions of 3β-Thiocyanato-14β-hydroxy-5β-card-20(22)-enolide

HANLEY N. ABRAMSON *‡, CHIAN L. HUANG *, THOMAS F. WU *, and THOMAS TOBIN ‡

Abstract □ The synthesis of a 3β-thiocyanatocardenolide is described. The compound exhibited about 0.1 times the cardiotonic effect of digitoxigenin in the isolated frog heart preparation. At a dosage of 20 mg/kg in the intact rat, it elicited ECG changes similar to those seen with a 10-mg/kg dose of digitoxigenin. Studies also revealed the new cardenolide to be a reversible inhibitor of sodium- and potassium-activated adenosine triphosphatase.

Keyphrases □ Thiocardenolides—3β-thiocyanato-14β-hydroxy-5β-card-20(22)-enolide synthesized, screened for cardiotonic activity and effect on sodium- and potassium-activated adenosine triphosphatase □ Cardiotonic agents, potential—3β-thiocyanato-14β-hydroxy-5β-card-20(22)-enolide synthesized and screened □ Adenosine triphosphatase, sodium and potassium activated—effect of 3β-thiocyanato-14β-hydroxy-5β-card-20(22)-enolide evaluated

Most naturally occurring cardioactive steroids are characterized by the presence of a free hydroxyl group at position 3β, as in digitoxigenin (I). While the changes in biological properties elicited by glycosidation, esterification, epimerization, and oxidation of the cardenolide 3β-hydroxyl group are well known and have been reviewed (1), nothing has been published concerning the effect of replacing the oxygen atom at position 3β by other heteroatoms such as nitrogen or sulfur.

The 3α- and 3β-aminodigitoxigenins were prepared (2), but their pharmacological actions were not described. Removal of the 3β-oxygen atom of I to give 3-deoxydigitoxigenin results in a compound that has cardiotonic activity on the isolated frog heart comparable to that of I (3, 4).

As part of a program to prepare and study the pharmacological actions of sulfur-containing cardenolides, the synthesis and biological properties of the 3β-thiocyanato analog (II) of I are now reported.

EXPERIMENTAL[1]

Chemistry—3β-Thiocyanato-14β-hydroxy-5β-card-20(22)-enolide (II) was prepared by a two-step sequence from 3-epidigitoxi-

[1] Melting points were taken on a Fisher-Johns melting-point stage and are uncorrected. UV absorption spectra were determined in 95% ethanol on a Beckman model DK2A recording spectrophotometer. IR absorption spectra were recorded on a Beckman model 8 recording spectrophotometer. NMR spectra were determined on a Varian EM 360 spectrometer. Microanalyses were conducted by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out with silica gel G. Column chromatography was carried out with silica gel 60 (Brinkmann 7734).