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Fluorescence Assay of Nitrofurantoin with *o*-Aminothiophenol in Plasma and Urine

NOBUTOSHI WATARI, TOMOO FUNAKI, and NOBUYOSHI KANENIWA ˣ

Received February 1, 1979, from the *School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo, 142, Japan.* Accepted for publication August 1, 1979.

Abstract □ A fluorescence method is presented for the determination of nitrofurantoin based on conversion of the drug to a fluorescent substance. The method requires 0.1–0.5 ml of plasma or diluted urine and is 10 times more sensitive than the commonly used colorimetric method.

Keyphrases □ Nitrofurantoin—fluorescence assay, plasma and urine □ Spectrophotofluorometry—analysis, nitrofurantoin, plasma and urine □ Antibacterials—nitrofurantoin, fluorescence assay, plasma and urine

Nitrofurantoin, 1-(5-nitro-2-furfurylideneamino)hydantoin (I), is used to treat urinary tract infections. The determination of drug levels in body fluids is important for pharmacokinetic and bioavailability studies. The content of I in plasma and urine commonly is determined by a colorimetric method based on the formation of a hyamine complex of I after its extraction into nitromethane (1, 2).

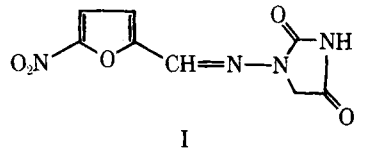
An electrochemical determination of I in urine that is faster and more accurate than the colorimetric method was reported (3). However, both methods require large sample volumes and are insufficiently sensitive for pharmacokinetic studies.

The reaction products obtained by heating some aromatic aldehydes with *o*-aminothiophenol (II) are fluorescent (4), and some drugs were assayed with II utilizing this reaction (5, 6). This paper discusses the application of this reaction to the fluorescence assay of I in plasma and urine.

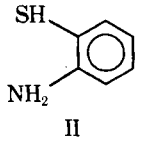
EXPERIMENTAL

Reagents—Nitrofurantoin[1] (I) was obtained commercially. *o*-Aminothiophenol hydrochloride was prepared by recrystallization of crude crystals obtained from a mixture of 1 ml of hydrochloric acid/g of *o*-aminothiophenol[2] (II) in ethanol (4). The other reagents were reagent grade.

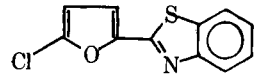
Procedure—Plasma, 0.1–0.5 ml, was deproteinized by mixing with 1 ml of ethanol. After centrifugation at 3000 rpm for 10 min, 0.5 ml of the supernate was transferred to a 15-ml test tube. Then 1 ml of 0.001% sodium nitrite, 2 ml of hydrochloric acid, 1 ml of 0.1% *o*-aminothiophenol hydrochloride, and 0.5 ml of distilled water were added. After mixing, the solution was heated at 70° in a water bath for 10 min. After cooling to room temperature, UV irradiation was carried out for 3 min; the relative fluoresence intensity was measured with excitation and emission at 375 and 422 nm, respectively.



I



II



III

The content of I in urine was determined similarly. If the urine was diluted eightfold or more with distilled water, 1 ml of the diluted urine could be assayed directly without deproteinization.

The spectrophotofluorometer[3] was standardized with quinine sulfate[4] in 0.1 *N* H_2SO_4.

The concentration of I in a sample was determined by comparing its fluorescence intensity with that of a standard solution prepared by adding a known concentration of I dissolved in ethanol to plasma or urine free of I and processing it in the same way as the test sample.

RESULTS AND DISCUSSION

A fluorescent substance, 2-(5-chloro-2-furyl)benzothiazole (III), was formed by the reaction of the 2-furaldehyde obtained by hydrochloric acid hydrolysis of I with II (6). However, the conversion of I to III varied due to a steric effect of the side chain of I (7) and incomplete replacement of the nitro group of 2-(5-nitro-2-furyl)benzothiazole with chlorine to give III (6). The nitro group can be replaced with chlorine by UV irradiation (6). The hydrolysis of I is reversible and is promoted by sodium nitrite, acetylacetone, formaldehyde, *etc.*

The maximum relative intensity of fluorescence was obtained in the presence of 0.001% sodium nitrite and with UV irradiation after heating. Therefore, these conditions were adopted for the assay of I with II. Typical results are summarized in Table I.

A linear relationship was obtained between the relative fluorescence intensity and the I concentration up to 5 μg/ml. The means of the relative fluorescence intensity of plasma samples varied slightly within a given analysis, but the standard deviations were similar. For this reason, it is necessary to run a standard I solution with each plasma and urine analysis. Under these conditions, quantitative measurements are possible.

To check the fluorescence method, plasma and urine samples from

[1] Fukuju Seiyaku Co.
[2] Tokyo Kasei Kogyo Co., Tokyo, Japan.
[3] Hitachi MPF-2A.
[4] Wako Pure Chemical Industries, Osaka, Japan.

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Table I—Fluorescence Method for Nitrofurantoin Determination (Relative Fluorescence Intensity per Microgram [a])

Sample[b]	Trial[c]	Nitrofurantoin Reacted, μg							
		0.25	0.50	0.75	1.00	5.00	10.00	Mean[d]	SD
Water (1.0 ml)	1	124.8	100.7	99.9	100.3	100.1	62.7	106.4	10.7
	2	95.1	100.3	99.1	94.1	109.2	90.0	99.6	6.0
	3	113.3	93.6	93.9	92.9	88.6	72.5	96.5	9.7
Rabbit plasma (0.1 ml)	1	109.5	95.1	95.8	108.3	94.1	84.0	100.6	7.6
	2	109.5	119.5	107.8	119.3	115.9	107.5	114.4	5.5
	3	75.9	88.3	79.5	85.0	84.5	64.8	82.6	4.9
Rabbit plasma (0.5 ml)	1				87.3				
	2				99.0				
	3				102.5				

[a] Blank values were subtracted. [b] Blank values for water and 0.1 and 0.5 ml of rabbit plasma were 7.8, 10.6, and 27.6 relative fluorescence intensity units, respectively. [c] $n = 3$. [d] The mean and standard deviation were calculated between 0.25 and 5 μg since the 10-μg values seemed unreliable.

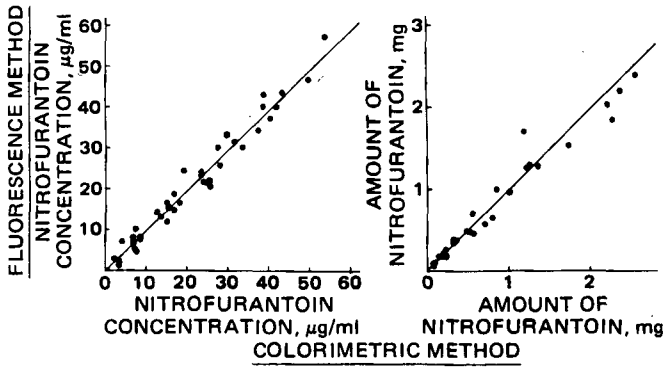

Figure 1—*Comparison of values obtained by the fluorescence and colorimetric methods for the determination of I in plasma (left) and urine (right).*

three rabbits dosed with I were assayed by the fluorescence and colorimetric methods. Figure 1 shows that good correlations were obtained for the values determined by the two methods in both plasma ($y = 0.980x + 0.125$, $r = 0.982$) and urine ($y = 0.980x - 0.026$, $r = 0.999$).

The fluorescence method for I is 10 times more sensitive than the colorimetric method, and it appears to be specific for I. It should be useful for the determination of microamounts of the drug for pharmacokinetic studies and other purposes.

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ACKNOWLEDGMENTS

The authors thank Professor T. Miyasaka and Dr. H. Arai, Showa University, for valuable advice.

Phenylfurans IV: Spasmolytic 3-Diethylamino-2,2-(dimethyl)propyl Esters of 5-Substituted Phenyl-2-furancarboxylic Acids

HOMER A. BURCH *x, RONALD E. WHITE *, GEORGE C. WRIGHT *, and MARVIN M. GOLDENBERG ‡

Received February 15, 1979, from the **Chemical Research Division and the* ‡*Biological Research Division, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication August 1, 1979.

Abstract □ A series of 3-diethylamino-2,2-(dimethyl)propyl 5-substituted phenyl-2-furancarboxylates was prepared and found to be pharmacologically active *in vitro* as GI tract nonanticholinergic smooth muscle spasmolytic agents. One of the more active compounds in the series contained the 5-(4-nitrophenyl) group.

Keyphrases □ Phenylfurans—3-diethylamino -2,2- (dimethyl)propyl esters of 5-substituted phenyl-2-furancarboxylic acids, synthesis, potential use as spasmolytic agents □ Spasmolytic agents—3-diethylamino-2,2-(dimethyl)propyl esters of 5-substituted phenyl-2-furancarboxylic acids, synthesis and pharmacological properties

The syntheses and pharmacological properties of 1- and 3-amino- and 1-amino-5-hydroxy-2,4-imidazolidinedione derivatives of 5-phenyl-2-furancarboxaldehydes have been described (1–3). This paper discusses the synthesis and *in vitro* pharmacological evaluation of a series of esters (I) derived from 3-diethylamino-2,2-(dimethyl)propanol (III) and 5-phenyl-2-furancarboxylic acids (II) (4). These compounds have potential use as spasmolytic agents (5).

DISCUSSION

Synthesis—The key intermediates in the synthesis of the esters were the appropriately substituted 5-phenyl-2-furancarboxylic acids (II). The acids generally were obtained in modest yields through the coupling of a substituted phenyldiazonium salt with 2-furancarboxylic acid. Several acids were prepared by oxidation of the corresponding 5-phenyl-2-fur-

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