# United States Patent

[11] 3,630,680



[72] Inventors **Walter Rittersdorf; Hans-Georg Rey; Peter Rieckmann, all of Mannheim-Waldhof, Germany**



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[73] Assignee **Boehringer Mannheim GmbH Mannheim, Germany**



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[33] **Germany**
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**July 3, 1968, Germany, No. P 17 67 931.1**


## [54] DIAGNOSTIC AGENTS FOR THE DETECTION OF UROBILINOGEN MATERIALS IN BODY FLUIDS

**22 Claims, No Drawings**


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[50] **Field of Search** ..... 23/230, 253 TP; 252/408



[56] **References Cited**

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| | | | |
|---|---|---|---|
| 3,378,346 | 4/1968 | Watson | 23/253 |
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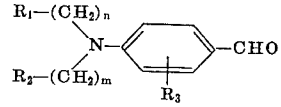
J. Fischl and N. Pinto, Clinica Chimica Acta, 2, 527– 33 (1957).



*Primary Examiner*—Morris O. Wolk
*Assistant Examiner*—Sidney Marantz
*Attorney*—Burgess, Dinklage & Sprung



**ABSTRACT:** Diagnostic agents for use in the detection of urobilinogen materials in body fluids such as urine, the agents comprising at least one strong acid and at least one compound which reacts with urobilinogen materials present in the body fluids to provide analytically measurable dyestuffs wherein said compound has the formula:



in which $R_1$ and $R_2$ which may be the same or different, each represent halogen, cyano, nitroso, carbalkoxy, carbamido, hydroxy, acyloxy, alkoxy, straight-chained and cyclic, saturated and unsaturated secondary and tertiary amino, or $R_1$ and $R_2$ together represent oxygen, sulfur, sulfonyl, alkylthionium, imino, alkylimino, substituted or unsubstituted arylimino or open-chained, alicyclic or heterocyclic dialkylimino, $R_2$ can also represent the residue of secondary straight or branched, open-chained or mono- or polycyclic alkyl or aralkyl and wherein one of $R_1$ and $R_2$ can also represent hydrogen, $R_3$ is hydrogen or alkyl, and *n* and *m* which may be the same or different each have a value of 0, 1, 2 or 3.

# DIAGNOSTIC AGENTS FOR THE DETECTION OF UROBILINOGEN MATERIALS IN BODY FLUIDS

The present invention relates to new and improved diagnostic agents for the detection of urobilinogen bodies in body fluids. More particularly, this invention relates to improved diagnostic agents for the detection of urobilinogen bodies in urine, spinal fluids and other body fluids.

It is known that urobilinogen bodies (bilans), indole, sulfonamides, porphobilinogens, urine indican and 5-hydroxy-indole-acetic acid can be detected using a solution of *p*-dimethylaminobenzaldehyde in hydrochloric acid. This detection test is known in the literature as Ehrlich's reaction. It has achieved considerable importance, particularly in medical diagnosis, for the detection of "increased urobilinogens" in the urine. Although the test is not very specific, it has come to be regarded as the standard test method for the diagnosis of diseases of both the liver and gall bladder.

In an attempt to simplify this test, it has already been proposed to make up test tablets (British Patent Specification No. 779,921) in which there is present *p*-dimethylaminobenzaldehyde, and a solid inorganic or organic acid (also U.S. Pat. No. 2,320,282 directed to test tablets for the detection of sulfonamides). When such tablets are dissolved in more or less diluted urine, there is obtained a color reaction which corresponds approximately to that produced in accordance with the original Ehrlich test. However, for the evaluation of the color reaction produced when, for example, sulfonamides are present, it is necessary to add a blue auxiliary dyestuff, such as methylene blue.

In accordance with a further proposal for the simplification of the Ehrlich test, the reaction has also been carried out employing test papers, (Fischl and Pinto, Clinica Chim. Acta, 2, 527–533/, and French Patent No. 1,450,273, in which the phosphoric acid used by Fischi and Pinto is replaced by oxalic acid).

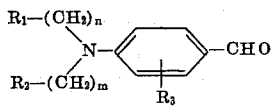
It is an object of the present invention to provide an improved diagnostic agent for use in the detection of unrobilinogens present in body fluids which avoids the disadvantages of the art.

Another object of the present invention is a simple and practical diagnostic agent for use in the detection of urobilinogens present in body fluids comprising a test paper or film.

Other objects will appear hereinafter.

In accordance with the invention, it has now been found that diagnostic agents for use in the detection of urobilinogens present in body fluids are obtained by employing a strong acid in admixture with a derivative of *p*-aminobenzaldehyde.

Preferred *p*-aminobenzaldehyde derivatives to be used according to the present invention are those which carry on the *p*-amino group, a branched alkyl group or a substituent which possesses strong electron attracting properties. Illustrative of the compounds of this type are those having the formula:




(I)

wherein $R_1$ and $R_2$, which may be the same or different, are each halogen, cyano, nitroso, carbalkoxy, carbamido, hydroxyl, acyloxy, alkoxy or open-chained or cyclic, saturated or unsaturated, secondary or tertiary amino or $R_1$ and $R_2$ together represent oxygen, sulfur, sulfonyl, alkylthionium, imino, alkylimino, unsubstituted or substituted arylimino open-chained, alicyclic or heterocyclic dialkylimino radical, $R_2$ can also be the residue of a secondary straight-chained or branched, open-chained, mono- or polycyclic alkyl or aralkyl and wherein one of the substituents $R_1$ and $R_2$ can be hydrogen, $R_3$ represents hydrogen or alkyl and *n* and *m*, which may be the same or different, are 0, 1, 2 or 3.



In preparing the diagnostic agents according to the invention, the compounds of the above formula (I) are preferably applied to an absorbent carrier, together with a strong, solid acid. The compounds (I) can also be used for the preparation of test film strips according to the procedure described in published Dutch Pat. application No. 67.15 828 and also in a form suitable for the detection of urobilinogens in solutions.

The preferred embodiment of the new diagnostic agents according to the present invention, i.e., test paper strips are prepared by impregnating an absorbent carrier, preferably filter paper, with a solution which contains 0.001–1 percent of an aldehyde of formula I and at least 3 percent, preferably 15–30 percent, of a strong, solid acid.

Examples of suitable strong, solid acids include sulfosalicyclic acid, p-toluene-sulfonic acid, potassium bisulfate, glutamic acid hydrochloride and preferably oxalic acid.

The impregnation solution can also contain inert additives, such as for instance polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of polyvinyl pyrrolidone and polyvinyl acetate, polyglycols and the like.

As solvents for use in preparing the solution for use in the impregnation of the absorbent carrier, there are suitable all readily volatile solvents in which the aldehydes (I) and also the acids are sufficiently soluble. Consequently, it is preferred to use polar solvents, such as methanol.

After impregnation, the filter papers are dried, cut up and, if desired, sealed on the synthetic resin foils or sealed between synthetic resin foils. When rodlets or strips are used as absorbent carriers then they are ready for use immediately after drying.

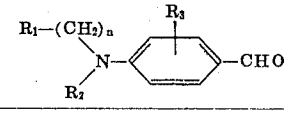
In carrying out the test reaction according to the present invention for the detection of urobilinogens or of unrobilinogen bodies, the new diagnostic agents according to the present invention are dipped into the solution to be investigated and the color change then evaluated after a short period of time, i.e., 1 to 2 minutes.

In contradistinction to the previously known diagnostic agents available for the detection of urobilinogens, the new diagnostic reagents according to the present invention are substantially more sensitive and are capable of detecting amounts as low as about 0.4 mg.-percent urobilinogen in urine.

Surprisingly, this color reaction is not disturbed by the presence of urea. Even a 3percent solution of urea hardly causes any color change to take place on the new test papers according to the present invention, whereas test papers which contain *p*-dimethylaminobenzaldehyde take on an intense yellow color.

Furthermore, it could not have been foreseen that the new diagnostic agents according to the present invention would not be adversely affected either by urine indican or by indole or by sulfonamides (in the case of normal dosages) in urine. This finding is particularly surprising because the compounds (I), dissolved in 2–20 percent hydrochloric acid, can be used very satisfactorily for the detection of indole in solution. For the detection of urobilinogens and of urobilinogen bodies in solutions, *p*-dimethylaminobenzaldehyde can, of course, be replaced by compounds of formula I and the content thus determined photometrically in a cuvette in a very exact manner and free of any interference.

A preferred group of benzaldehyde derivatives according to the present invention are compounds having the formula:




(II)

wherein $R_1$ is hydrogen, halogen, cyano, nitroso, carbalkoxy carbamido, hydroxyl, acyloxy, alkoxy, open-chained or cyclic, saturated or unsaturated, secondary or tertiary amino, $R_2$ is the residue of a secondary, straight-chained, branched, open-

chained or mono- or polycyclic alkyl or aralkyl radical, $R_3$ is hydrogen or alkyl and *n* is 0, 1, 2 or 3.

Thus according to one aspect of the present invention, there is provided a diagnostic agent for the detection of urobilinogen bodies in body fluids, particularly in urine, which comprises an absorbent carrier or a film, a strong, solid acid and a substance which, in the manner of the Ehrlich test, produces a color change in the presence of urobilinogen bodies, characterized in that as color-producing substance there is used at least one compound of formula I.

According to a further aspect of the present invention, there is provided a process for the detection of urobilinogen bodies present in body fluids, the process being carried out through the use of a test strip, i.e., an absorbent carrier or, a reagent film or in solution in the presence of a strong, solid or liquid acid and a substance which, in the manner of Ehrlich's test, produces a color change in the presence of urobilinogen bodies, characterized in that a compound of formula I is used as the color indicator.

For a fuller understanding of the nature and objects of this invention, reference may be had to the following examples, which are given merely as further illustrations of the invention and are not to be construed in a limiting sense.

## EXAMPLE 1

Filter paper (Schleicher & Schüll No. 2316) was impregnated with a solution having the following composition:

| | |
|---|---|
| *p*-bis-(β-diethylaminoethyl)-aminobenzaldehyde | 0.2 g. |
| oxalic acid | 20.0 g. |
| methanol | ad 100.0 ml. |

Following drying of the impregnated paper, there was obtained a white test paper which, with normal urine, gave a pale pink color and with urine specimens which contained more than 0.4 mg.-percent of urobilinogen bodies gave, depending upon the content of urobilinogen therein, a more or less intensive pink to violet color. Urine specimens free of urobilinogen bodies, as well as 3 percent aqueous solutions of urea, did not bring about any color change in the test paper. Urine indican also did not produce a color reaction. The test papers could be evaluated after 1–2 minutes following wetting with the liquid samples to be tested.

## EXAMPLE 2

Filter paper (Schleicher & Schüll No. 2316) was impregnated with a solution having the following composition:

| | |
|---|---|
| p-bis-(β-cyanoethyl)-aminobenzaldehyde | 0.05 g. |
| oxalic acid | 20.0 g. |
| polyvinyl alcohol | 3.0 g. |
| water | 30.0 ml. |
| methanol | ad 100.0 ml. |

Following drying of the impregnated paper there was obtained a white test paper having the same properties as that produced according to example 1.

## EXAMPLE 3

Filter (Schleicher & Schüll No. 2316) was impregnated, according to the procedure described in example 1, using various concentrated solutions containing *x* parts of p-N-(N'-methyl-piperazino)-benzaldehyde (MPBA) or of p-dimethylaminobenzaldehyde (DABA) and 20 parts of oxalic acid in 100 parts of methanol. All of the test papers thusly obtained were white following drying. The dried test papers were dipped either into a 3 percent solution of urea or into a urine sample which was free of urobilinogen. The color changes which resulted are set out in the following table:

TABLE I

| X | MPBA | DABA |
|---|---|---|
| 0.05 | white | yellow |
| 0.075 | white | yellow |
| 0.1 | white | yellow |
| 0.2 | pale yellowish | strongly yellow |

As can be clearly seen from the above results, the test paper according to the present invention, in contradistinction to the known test paper, is substantially insensitive to urea and thus reacts more sensitively and more specifically with urobilinogen bodies as the pink color reaction with urobilinogen is not masked by the more or less strongly yellow color reaction with urea. It is, of course, obvious that such a yellow color reaction considerably impairs the sensitivity and measurability of the urobilinogen reaction.

## EXAMPLE 4

Filter paper (Schleicher & Schüll No. 23S or 2316) was impregnated with methanolic solutions which, per 100 parts of solution, contained A parts of the compounds I as given in the following table and B parts of oxalic acid. White test papers were thereby obtained in each case.

The color reactions which resulted following immersion of these test papers into various solutions were evaluated after 1–2 minutes. The results are set out in table II which follows:

TABLE II

| Compound (I) | A | B | Color reaction with— Urobilin ogen-free urine=3% urea soln. | Normal urine | Urobilin ogen-containing urine |
|---|---|---|---|---|---|
| p-N,N'-dimethyl-N'-ethylhydrazino-benzaldehyde | 0.1 | 25 | Yellowish | Yellow-pink | Red-violet. |
| p-N-nitroso-N-methylaminobenzaldehyde | 0.1 | 25 | do | Yellowish | Orange. |
| p-N-cyanomethyl-N-methylamino-benzaldehyde | 0.1 | 25 | White | Pale pink | Pink-red. |
| p-bis-(carbethoxymethyl)-aminobenzaldehyde | 0.1 | 25 | do | do | Do. |
| p-N-β-chloroethyl-N-methylaminobenzaldehyde | 0.1 | 25 | Yellowish | Yellow-pink | Purple. |
| p-N-β-hydroxyethyl-N-methylaminobenzaldehyde | 0.1 | 25 | do | do | Do. |
| p-N-β-methoxyethyl-N-methylaminobenzaldehyde | 0.1 | 25 | do | do | Do. |
| p-bis-(β-fluoroethyl)-amino-benzaldehyde | 0.1 | 25 | Pale yellowish | Pale pink | Blue-red. |
| p-bis-(β-chloroethyl)-amino-benzaldehyde | 0.1 | 25 | do | do | Do. |
| p-bis-(β-bromoethyl)-aminobenzaldehyde | 0.1 | 25 | do | do | Do. |
| p-bis(β-hydroxyethyl)-aminobenzalehyde | 0.1 | 20 | Yellowish | Yellow-pink | Purple. |
| p-bis(β-methoxyethyl)-amino-benzaldehyde | 0.1 | 20 | do | do | Do. |
| p-bis-(β-cyanoethyl)-amino-benzaldehyde | 0.15 | 25 | White | Pale pink | Pink-red. |
| p-bis-(β-dimethylaminoethyl)-aminobenzaldehyde | 0.2 | 20 | do | do | Do. |
| p-bis(β-diethylaminoethyl)-aminobenzaldehyde | 0.2 | 20 | do | do | Do. |
| p-bis-(β-N-piperidinoethyl)-aminobenzaldehyde | 0.1 | 20 | do | do | Do. |
| [p-bis-(β-triethylammoniumethyl)-aminobenzaldehyde]dibromide | 0.2 | 20 | do | do | Do. |
| [p-bis-(N-pyridiniumethyl)-aminobenzaldehyde]dibromide | 0.2 | 20 | do | do | Do. |
| p-N-morpholino-benzaldehyde | 0.1 | 20 | Yellowish | Yellow-pink | Red-violet. |
| p-N-thiomorpholino-benzaldehyde | 0.1 | 20 | do | do | Do. |
| [p-N-(S-methylthio-morpholinium)-benzaldehyde] iodine | 0.2 | 20 | White | Pale pink | Pink-red. |
| p-N-(S-dioxothio-morpholino)-benzaldehyde | 0.1 | 25 | do | do | Do. |
| [p-bis-(N-quinolinium-ethyl)amino-benzaldehyde] dibromide | 0.2 | 20 | do | do | Do. |
| p-N-piperazinobenzaldehyde | 0.1 | 20 | do | do | Do. |
| p-N-(N'-methylpiperazino)-benzaldehyde | 0.05 | 20 | do | do | Do. |
| p-N-[N'-(p-formyl-phenyl)-piperazino]-benzaldehyde | 0.1 | 25 | Pale yellowish | do | Red-violet. |
| [p-N-(N'-dimethyl-piperazinum)-benzaldehyde]-iodine | 0.1 | 20 | White | do | Pink-red. |

TABLE II—Continued

| Compound (I) | A | B | Color reaction with— Urobilin ogen-free urine=3% urea soln. | Normal urine | Urobilin ogen-containing urine |
|---|---|---|---|---|---|
| [p-N-(N'-cyclepentamethylenepiperazinium)-benzaldehyde]-iodine | 0.2 | 20 | do | do | Do. |
| p-N-[N'-cyclo-(3-oxapentamethylene)-piperazinium]-benzalhehyde iodine | 0.2 | 20 | do | do | Do. |
| p-bis-(carbethoxy-ethyl)-amino-benzaldehyde | 0.1 | 20 | Yellowish | Yellowish pink | Red. |
| p-bis-(carbamido-ethyl)-aminobenzaldehyde | 0.1 | 20 | Yellowish | Yellow-pink | Red. |
| p-bis-(γ-bromopropyl)-aminobenzaldehyde | 0.05 | 20 | Pale-yellowish | Pale pink | Pink-red. |
| p-bis-(γ-cyanopropyl)-aminobenzaldehyde | 0.05 | 20 | do | do | Do. |
| p-bis-(γ-dimethyl-aminopropyl)-aminobenzaldehyde | 0.05 | 20 | do | do | Do. |
| [p-bis-(γ-trimethyl-ammoniumpropyl)-aminobenzaldehyde]-dichloride | 0.05 | 20 | White | do | Do. |
| p-N-diethylamino-ethyl-N-methylaminobenzaldehyde | 0.05 | 25 | Pale yellowish | do | Purple. |
| p-N-dimethylamino-ethyl-N-methylaminobenzaldehyde | 0.05 | 25 | do | do | Do. |
| p-N-diisopropylaminoethyl-N-methylaminobenzaldehyde | 0.05 | 25 | do | do | Do. |
| p-N-diethylaminoethyl-N-ethylaminobenzaldehyde | 0.05 | 25 | do | do | Do. |
| p-(1-methyl-1,5-diazacyclooctyl-5)-benzaldehyde | 0.05 | 25 | do | do | Pink-red. |
| p-N-cyanoethyl-N-methylaminobenzaldehyde | 0.05 | 25 | Yellowish | do | Purple. |
| p-N-cyanoethyl-N-ethylaminobenzaldehyde | 0.05 | 25 | do | Yellowish pink | Do. |
| p-N-γ-dimethylaminopropyl-N-methyl-aminobenzaldehyde | 0.05 | 25 | Yellow | Yellow-pink | Do. |
| p-N-bis-sulfoethylaminobenzaldehyde | 0.05 | 25 | Yellowish | Pale pink | Pink-red. |
| p-N-diethylamino-ethyl-N-isopropylamino-benzaldehyde | 0.05 | 25 | do | do | Purple. |
| p-N-cyanoethyl-aminobenzaldehyde | 0.01 | 20 | White | do | Pink-red. |
| p-N-diethylamino-ethylaminobenzaldehyde | 0.05 | 20 | Pale yellowish | do | Do. |

5 Strips of filter paper (Schleicher & Schüll No. 2316) were impregnated either with a solution of 0.05 g. p-N-(N'-methyl-piperazino)-benzaldehyde (MPBA) or of p-dimethylaminobenzaldehyde (DABA) and 20 g. oxalic acid in 100 ml. methanol and then dried.

The dried papers were then immersed into aqueous solutions of conventionally used sulfonamides and evaluated after 1–2 minutes. The color changes which were observed are set out in table III which follows:

TABLE III

| Indicator | Sulfonamide | 10 mg. percent | 25 mg. percent | 50 mg. percent |
|---|---|---|---|---|
| MPBA | Sulfametin | White | White | Pale yellowish. |
| | Sulfafurazole | do | do | Do. |
| | Sulfaphenazole | do | do | White. |
| | Sulfamoxolum | do | Pale yellowish | Yellowish. |
| DABA | Sulfametin | Yellowish | Yellow | Strong yellow. |
| | Sulfafurazole | Yellow | Strong yellow | Very strong yellow. |
| | Sulfaphenazole | White | Pale pink | Pale pink. |
| | Sulfamoxolum | Yellow | Strong yellow | Very strong yellow. |

sulfametin = N¹-(5-methoxy-2-pyrimidinyl)-sulfanilamide
sulfafurazole = N¹-(3,4-dimethyl-5-isoxazolyl)-sulfanilamide
sulfaphenazole = N¹-(1-phenyl-5-pyrazolyl)-sulfanilamide
sulfamoxolum = N¹-(4,5-dimethyl-2-oxazolyl)-sulfanilamide

As can be seen from the table, the color reaction with urobilinogens, using the test strips according to the present invention, is scarcely affected by sulfonamide concentrations of up to 50 mg.-percent. Higher concentrations only occur and then for a brief period of time following the administration of a massive dose for "shock" therapy and are, therefore, usually known to the observer.

## EXAMPLE 6

For use in the production of a reagent film suitable for the detection of urobilinogens, there was prepared a mixture of the following components:

| | |
|---|---|
| p-bis-(β-cyanoethyl)-aminobenzaldehyde | 0.75 g. |
| syrupy orthophosphoric acid | 5.00 g. |
| colloidal silicic acid ("Aerosil") | 6.00 g. |
| organic sodium sulfonate (rapid wetting agent) | 2.00 g. |
| polyvinyl butyracetal ("Mowital") | 10.00 g. |
| polyethylene glycol 6,000 | 10.00 g. |
| methanol | 100.0 ml. |

There was applied a 300 μthick layer of the above mixture onto a white polyvinylchloride foil, followed by drying with warm air. A water-insoluble film was thereby formed. When this was moistened with a drop of urobilinogen-containing urine, after 1–2 minutes had elapsed there was observed a red color which, following wiping off the urine, became darker. No coloration was observed when urine free of urobilinogen was tested in the same manner.

## EXAMPLE 7

25 ml. urine were acidified with 12 ml. glacial acetic acid and extracted with 40 ml. ether. The extract was washed twice with 20 ml. amounts of water and again made up to 40 ml. with ether. 20 ml. of this extract was mixed with 0.5 ml. of a solution of 1 g. p-N-morpholino-benzaldehyde in 5 ml. concentrated hydrochloric acid and the resultant mixture shaken vigorously. Thereafter, there were added 6 ml. of a semisaturated solution of sodium acetate, followed by shaking up again. The red material which was formed thereby collected in the aqueous phase. After separation, the ether phase was again washed with 2 ml. water. The combined aqueous extracts were made up to 20 ml., the color present measured in a photometer at 557 nm. and the result evaluated by means of a previously prepared calibration curve.

## EXAMPLE 8

Filter paper (Schleicher & Schüll No. 2316) was impregnated with a solution of 0.5 parts pbis-(diethylaminoethyl)-aminobenzaldehyde bis-hydrogen oxalate, 30 parts maleic acid and 0.5 parts polyethylene glycol 1,000 in 100 parts methanol, and then dried.

Strips of this paper reacted with urobilinogen-containing urine to provide a pink to red color.

Papers with the same analytical properties but which were easier to unroll and cut up were obtained by additionally using 5 parts polyethylene glycol 6,000 or 20,000.

## EXAMPLE 9

Filter paper (Schleicher & Schüll No. 2316) was impregnated with a solution having the following composition:

| | |
|---|---|
| p-(cyclohexylamino)-benzaldehyde | 0.05 g. |
| oxalic acid | 20.0 g. |
| methanol | ad 100.0 ml. |

After the impregnated paper was dried there was obtained a white test paper which, when moistened with urine samples containing urobilinogen bodies, gave a more or less intensive pink-to-violet coloration depending on the urobilinogen con-

centration. Urine samples having a very low content of urobilinogen bodies and 3 percent aqueous solutions of urea did not give any color change in the test paper. Urine indican also gave no color reaction. The test papers can be evaluated for color change after 1–2 minutes.

## EXAMPLE 10

Filter paper (Schleicher & Schüll No. 2316) was impregnated with a solution having the following composition:

| | |
|---|---|
| *p*-(sec.-butylamino)-benzaldehyde | 0.05 g. |
| potassium bisulfate | 15.0 g. |
| polyethylene glycol | 5.0 g. |
| water | ad 100.0 ml. |

After drying the impregnated paper, there was obtained a white test paper which had the same properties as the test paper produced according to example 9.

## EXAMPLE 11

Strips of filter paper (Schleicher & Schüll No. 2316) were impregnated with solutions having various concentrations containing X parts *p*-(isopropylamino)-benzaldehyde (iPABA), *p*-(*n*-propylamino)-benzaldehyde (nPABA) or *p*-(dimethylamino)-benzaldehyde (DABA) and 20 parts oxalic acid in 100 parts methanol, according to the procedure described in Example 1. The dried test papers were dipped into 3% solutions of urea. The color changes which were thereby observed are set out in the following Table:

Table IV

| X | iPABA | nPABA | DABA |
|---|---|---|---|
| 0.05 | white | yellowish | yellow |
| 0.075 | white | yellowish | yellow |
| 0.1 | white | yellow | yellow |
| 0.15 | pale yellowish | yellow | strong yellow |

As can be clearly seen from the results set out in the above table, the test papers according to the present invention which contained p-(isopropylamino)-benzaldehyde were substantially less sensitive to urea and thus reacted more sensitively and specifically with urobilinogen bodies as the pink color reaction with urobilinogen was not masked by the more or less strong yellow color reaction with urea.

## EXAMPLE 12

Filter paper (Schleicher & Schüll No. 23S or 2316) was impregnated with methanolic solutions which contained, in 100 parts of solution, 0.05 parts of the compounds I which are set out in the following table and 20 parts oxalic acid. The test papers which were obtained were white to yellow in appearance.

Upon dipping these test papers into the liquids described in the table, there were observed, after 1–2 minutes, the noted colors. The results which were obtained are set out in table V which follows:

TABLE V

| Compound (I) | Reaction with 3% urea soln. or with urine of low urobilinogen content | Reaction with urobilinogen-containing urine |
|---|---|---|
| p-(isopropylamino)-benzaldehyde | White * | Pink-red. |
| p-(isopropylamino)-m-methyl benzaldehyde. | Pale yellowish | Do. |
| p-(sec.-butylamino)-benzaldehyde | do | Do. |
| p-(pentyl-3-amino)-benzaldehyde | Yellowish | Do. |
| p-(2,4-dimethylpentyl-3-amino)-benzaldehyde. | Yellow | Red. |
| p-(cyclopentylamino)-benzaldehyde | Pale yellowish. | Red-violet. |
| p-(2-methyl-cyclopentylamino)-benzaldehyde. | Yellowish | Do. |
| p-(cyclohexylamino)-benzaldehyde | Almost white * | Do. |
| p-(cyclohexylamino)-m-methyl-benzaldehyde. | Pale yellowish. | Do. |
| p-(cyclohexylamino)-m-isopropyl-benzaldehyde. | Yellowish | Do. |
| p-(2-methyl-cyclohexylamino)-benzaldehyde. | do | Do. |
| p-menthylaminobenzaldehyde | Yellow | Orange. |
| p-(1-cyclohexyl-propyl-2-amino)-benzaldehyde. | Yellowish | Pink-red. |
| p-(cyclododecylamino)-benzaldehyde | Pale yellowish. | Pink. |
| p-(norbornyl-2-amino)-benzaldehyde | Yellow | Pink-red. |
| p-(α-methyl-benzylamino)-benzaldehyde. | Yellowish | Do. |
| p-(α-isopropyl-benzylamino)-benzaldehyde. | do | Do. |
| p-(1-phenyl-propyl-2-amino)-benzaldehyde. | do | Do. |
| p-(indanyl-2-amino)-benzaldehyde | do | Do. |
| p-(N-diethylaminoethyl-N-isopropyl-amino)-benzaldehyde. | do | Purple. |

* The strips dipped into urine had a weak yellow coloration due to the inherent color of the urine.

## EXAMPLE 13

For the production of a filmlike reagent layer for use in the detection of urobilinogens, there was prepared a mixture of the following components:

| | |
|---|---|
| p-(cyclohexylamino)-benzaldehyde | 0.05 g. |
| colloidal silicic acid ("Aerosil") | 1.00 g. |
| oxalic acid | 10.00 g. |
| polyvinyl butyracetal ("Mowital") | 0.5 g. |
| polyethylene glycol 6000 | 0.5 g. |
| methanol | 30.0 ml. |

The above mixture was then applied at a thickness of about 1 mm. to a white polyvinylchloride foil and thereafter allowed to dry in warm air. A water-insoluble layer was thereby formed. When this was moistened with a drop of urobilinogen-containing urine then, following the passing of 1–2 minutes, there was observed a red coloration. No coloration was observed with a urobilinogen-free urine.

## EXAMPLE 14

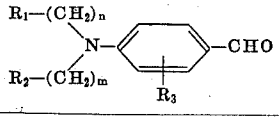
25 ml. urine were acidified with 12 ml. glacial acetic acid and extracted with 40 ml. ether. The extract was washed twice with 20 ml. amounts of water and again made up to 40 ml. with ether. 20 ml. of the thusly obtained extract were mixed with 0.5 ml. of a solution of 1 g. p-(cyclopentyl-amino)-benzaldehyde in 5 ml. concentrated hydrochloric acid and the mixture shaken vigorously. Thereafter, there were added 2.5 ml. of a semisaturated solution of sodium acetate and 3.5 ml. water, following which the mixture was again shaken, the red colored material thereby formed collected in the aqueous phase. After separation, the ether was again washed with 2 ml. water. The combined aqueous extracts were made up to 20 ml. and the color thereof measured in a photometer at 557 nm. The reading obtained was evaluated by means of a previously prepared calibration curve.

It is a general advantage of those substances I which possess branched alkyl substituents at the amino group, that they do not react with unknown ingredients of certain urine samples. Further, it could not have been foreseen, that the more basic derivatives of *p*-aminobenzaldehyde having a branched alkyl substituent $R_2$ at the amino group would not react with urea, although up until now it had been found that more basic aldehydes do react better with urea.

We claim:

1. Diagnostic agent for the detection of urobilinogen bodies in body fluids comprising at least one strong acid and at least one compound which reacts with urobilinogen bodies to pro-

vide an analytically measurable dyestuff, said compound having the formula:



wherein

A. $R_1$ and $R_2$ are each members selected from the group consisting of halogen, cyano, nitroso, carbalkoxy, carbamido, hydroxy, acyloxy, alkoxy and amine radicals having at least one aliphatic hydrocarbon substituent and, if there are two aliphatic hydrocarbon substituents they may, together with the amine nitrogen, represent an N-aliphatic heterocycle and wherein one of $R_1$ and $R_2$ can represent hydrogen.

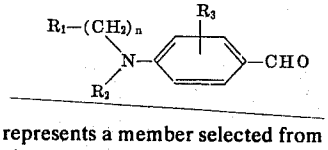
B. $R_1$ and $R_2$ together can represent a member selected from the group consisting of oxygen, sulfur, sulfonyl, alkylthionium, imino, alkylimino, substituted or unsubstituted arylimino, open-chained dialkylimino, cyclic dialkylimino and heterocyclic dialkylimino;

C. R-($CH_2$) can also represent a member selected from the group consisting of secondary straight-chained alkyl, branched-chained alkyl, monocyclic alkyl, polycyclic alkyl and aralkyl with the proviso that in this case, $R_1$ can represent hydrogen only if *n* is 0;

$R_3$ is a member selected from the group consisting of hydrogen and alkyl; and

*n* and *m* are each one of 0, 1, 2 and 3; and a carrier therefor.

2. Diagnostic agent according to claim 1 wherein said compound which reacts with urobilinogen bodies to provide an analytically measurable dyestuff has the formula:



wherein $R_1$ represents a member selected from the group consisting of hydrogen, halogen, cyano, nitroso, carbalkoxy, carbamido, hydroxy, acyloxy, alkoxy and amine radicals having at least one aliphatic hydrocarbon substituent and, if there are two aliphatic hydrocarbon substituents they may, together with the amine nitrogen, represent an N-aliphatic heterocycle, $R_2$ represents a member selected from the group consisting of secondary straight-chained alkyl, branched-chained alkyl, monocyclic alkyl, polycyclic alkyl and aralkyl, $R_3$ is a member selected from the group consisting of hydrogen and alkyl and *n* is 0, 1, 2 and 3, with the proviso that $R_1$ is hydrogen only when *n* is 0.

3. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution containing 0.001–1 percent of a compound (I) as defined in claim 1 and 15–30 percent of a strong solid acid.

4. Diagnostic agent according to claim 1 wherein said strong acid is a member selected from the group consisting of sulfosalicyclic acid, p-toluene-sulfonic acid, sulfuric acid, glutamic acid, *o*-phosphoric acid, oxalic acid, hydrochloric acid and maleic acid.

5. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition:

| | |
|---|---|
| *p*-bis-(β-diethylaminoethyl)-aminobenzaldehyde | 0.2 g. |
| oxalic acid | 20.0 g. |
| methanol | ad 100.0 ml. |

6. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition:

| | |
|---|---|
| p-bis-(β-cyanoethyl)-aminobenzaldehyde | 0.05 g. |
| oxalic acid | 20.0 g. |
| polyvinyl alcohol | 3.0 g. |
| water | 30.0 ml. |
| methanol | ad 100.0 ml. c |

7. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition: 0.05 and 0.2 parts of a member selected from the group consisting of p-N-(N'-methyl-piperazino)-benzaldehyde and p-dimethylaminobenzaldehyde and 20 parts of oxalic acid in 100 parts of methanol.

8. Diagnostic agent according to claim 1 wherein said carrier is a polyvinylchloride film having applied thereon a layer of the following composition:

| | |
|---|---|
| p-bis-(β-cyanoethyl)-aminobenzaldehyde | 0.75 g. |
| syrupy orthophosphoric acid | 5.00 g. |
| colloidal silicic acid ("Aerosil") | 6.00 g. |
| organic sodium sulfonate (rapid wetting agent) | 2.00 g. |
| polyvinyl butyracetal ("Mowital") | 10.00 g. |
| polyethylene glycol 6000 | 10.00 g. |
| methanol | 100.00 ml. |

9. Diagnostic agent according to claim 1 wherein said carrier is a liquid containing p-N-morpholinobenzaldehyde in solution in concentrated hydrochloric acid.

10. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition: 0.5 parts p-bis-(diethylaminoethyl)-aminobenzaldehyde bis-hydrogen oxalate, 30 parts maleic acid and 0.5 parts polyethylene glycol 1,000–20,000 in 100 parts methanol.

11. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition:

| | |
|---|---|
| p-(cyclohexylamino)-benzaldehyde | 0.05 g. |
| oxalic acid | 20.0 g. |
| methanol | ad 100.0 ml. |

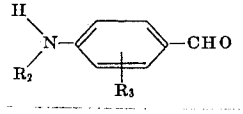
12. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution having the following composition:

| | |
|---|---|
| p-(sec.-butylamino)-benzaldehyde | 0.05 g. |
| potassium bisulfate | 15.0 g. |
| polyethylene glycol | 5.0 g. |
| water | ad 100.0 ml. |

13. Diagnostic agent according to claim 1 wherein said carrier is a polyvinylchloride film having applied thereon a layer of the following composition:

| | |
|---|---|
| c p-(cyclohexylamino)-benzaldehyde | 0.05 g. |
| colloidal silicic acid ("Aerosil") | 1.00 g. |
| oxalic acid | 10.00 g. |
| polyvinyl butyracetal ("Mowital") | 0.5 g. |
| polyethylene glycol 6000 | 0.5 g. |

14. Diagnostic agent as claimed in claim 1 wherein said compound has the formula



wherein $R_2$ is a secondary alkyl group which can be straight-chained, branched or cyclic and wherein $R_3$ is a member selected from the group consisting of hydrogen and alkyl.

15. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface.

16. A method for carrying out an analytical test for the presence of urobilinogen bodies in biological fluids which comprises applying to the wettable surface of a diagnostic agent according to claim 15 a small amount of the fluid to be tested and examining said paper for visual evidence of the presence of said urobilinogen bodies.

17. Diagnostic agent according to claim 1 wherein said carrier is a foil.

18. A method for carrying out an analytical test for the presence of urobilinogen bodies in biological fluids which comprises applying to the coated surface of a diagnostic agent according to claim 17 a small amount of the fluid to be tested and examining said coating for visual evidence of the presence of said urobilinogen bodies.

19. Diagnostic agent according to claim 1 wherein said carrier is a liquid. open-chajned, alicyclic or heterocyclic dialkt54

20. A method for carrying out an analytical test for the presence of urobilinogen bodies in biological fluids which comprises admixing a diagnostic agent according to claim 19 with a small amount of an ether extract of the fluid to be tested, adding a semisaturated solution of sodium acetate and water and examining the resultant aqueous phase for visual evidence of the presence of urobilinogen bodies.

21. Diagnostic agent according to claim 1 wherein said carrier is a paper sheet having a wettable surface impregnated with a solution containing 0.001 to 1 percent of a compound (I) as defined in claim 1 and at least 3 percent of a strong solid acid.

22. Diagnostic agent according to claim 21 additionally containing at least one member selected from the group consisting of polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of polyvinyl pyrrolidone and polyvinyl acetate and polyglycols.

* * * * *

# UNITED STATES PATENT OFFICE

# CERTIFICATE OF CORRECTION

Patent No. 3,630,680 Dated December 28, 1971

Inventor(s) Walter Rittersdorf et al.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 1, line 22

"tablet," should be --tablets--

Col. 1, line 34

After "533/" insert --1957--

Col. 1, line 38

"unro-" should be --uro- --

Col. 2, line 32

"unrobilinogen" should be --urobilinogen--

Col. 3, line 2

"nis" should be --n is--

Col. 5, line 20

Before line 21, insert --EXAMPLE 5--

line 21, delete "5"

Col. 6, line 53

"pbis" should be --p-bis--

Col. 10, Claim 13

1st line of composition ingredients, delete "c" before "p"



USCOMM-DC 60376-P69
* U.S. GOVERNMENT PRINTING OFFICE : 1969 O—366-334.

# UNITED STATES PATENT OFFICE
# CERTIFICATE OF CORRECTION

Patent No. 3,630,680 Dated December 28, 1971

Inventor(s) Walter Rittersdorf et al. 

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 10, Claim 13, add to the ingredient listing, as a 6th line -- methanol 30.0 ml. --. Column 11, Claim 19 should read -- 19. Diagnostic agent according to claim 1 wherein said carrier is a liquid.

Signed and sealed this 7th day of November 1972.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

ROBERT GOTTSCHALK
Commissioner of Patents

FORM PO-1050 (10-69)

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