J. Pharm. Pharmac., 1966, **18**, 112–114 Received December 11, 1964

Determination of micro amounts of 3-(dithien-2-ylmethylene)-1-methylpiperidine, a non-narcotic antitussive

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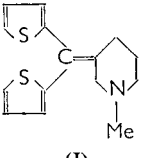
A specific colorimetric method for the determination of micro amounts of 3-(dithien-2-ylmethylene)-1-methylpiperidine, a new antitussive and expectorant, is described. Depending on amount, a characteristic colour varying from rose pink to deep bluish purple appears when 2 volumes of a 0·01% solution of ninhydrin in concentrated sulphuric acid are added to 1 volume of an aqueous solution of the drug. After 24 hr at room temperature the colour produced has an absorption maximum at 590 mμ and follows Beer's Law over the range 1–25 μg/ml. The colour is specific for the drug and is sensitive at the μg/ml level. Recoveries from aqueous solution and human urine were 97·4 ± 2·1 and 97·4 ± 1·7% respectively.

A NEW kind of potent and non-narcotic antitussive drug [3-(dithien-2-ylmethylene)-1-methylpiperidine; AT-327, Asverin; I] with expectorant activity has been described (Kasé, Yuizono, Yamasaki, Yamada, Io, Tamiya & Kondo, 1959; Sugimoto, Kowa, Higaki, Nakamura & Yasaka, 1960). In the absence of a method suitable for quantitative determination of the drug in pharmaceutical preparations and biological materials, a sensitive colorimetric method has been devised by us.*

The drug as citrate and hydrochloride, exhibits two ultraviolet absorption peaks in an aqueous solution, the higher at 208 and the other at 247 mμ. The absorption at 247 mμ follows Beer's Law and is suitable for



(I)

quantitative determination of the drug over the range of 1 to 25 μg/ml. However, it is unsuitable for the determination of the drug in biological materials such as urine, because many substances show similar absorption maxima in the neighbourhood of this wavelength.

According to Feigl (1960), thiophen, and its derivatives unsubstituted in the α-position, condense with 1,2-diketones such as benzil, isatin, and ninhydrin in concentrated sulphuric acid to yield coloured quinoidal compounds. Of these, 0·01% ninhydrin solution in concentrated sulphuric acid provides the most sensitive test for thiophen itself (limit of identification: 0·2 μg).

The reaction just described has been adapted to the determination of the new drug, and recoveries from aqueous solution and human urine have been investigated.

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* Abstract of a paper was read at the Meeting of Japanese Pharmacological Society at Kyushu University on November 2, 1960.

Experimental

METHOD

This incorporates the optimum reaction conditions, selected after examining the effects of the ratio of mixing of reagent and drug, concentration of acid and ninhydrin in the acid, and time and temperature as applied to the citrate. No significant differences were observed with other salts examined.

Reagent. 0·01% ninhydrin-sulphuric acid solution. Dissolve 10 mg of ninhydrin in 100 ml of pure concentrated sulphuric acid, thiophen-free. The reagent must be freshly prepared.

Standard solution of drug. Dissolve 169·0 mg of 3-(dithien-2-ylmethylene)-1-methylpiperidine citrate [white needles, m.p. 135° (decomp.)] in 1 litre of distilled water to give a 0·1 mg/ml of drug calculated as base. Dilute the solution further as required.

Procedure. Pipette 1·5 ml of the test solution into a small test tube, and add ninhydrin reagent (3·0 ml) slowly with ice-cooling and thorough mixing. Develop the colour by allowing the mixture to stand at 20–25° for 24 hr in the dark. Read the absorbance at 590 mμ using a spectrophotometer. For a blank, use a mixture of one part of distilled water and two parts of ninhydrin reagent.

SPECIFICITY

In testing for the specificity of the reaction, both the colour and absorption maximum of the solution yielded by the drug and those given by reaction solutions of similar compounds possessing either one thiophen or two thiophen nuclei were investigated under the experimental conditions described. 25 compounds were examined. These were: thiophen; four derivatives closely related to AT-327; thiambutene and five derivatives; two tertiary amine derivatives of ethyl 2-aminomethyl-3,3-di-thien-2′-yl-acrylates; 1,2,5,6-tetrahydro-1-methyl-4-thien-2′-yl-pyridine; 1-methyl-3-α-thiophenoyl piperidine and two derivatives; seven tertiary amine derivatives of 2-(*N*-aminoalkylacylamido)thiophen; 2-[*N*-ethyl-*N*-(2-piperidinopropyl)carbamoyl]thiophen.

Each compound yielded with the ninhydrin reagent a solution having a characteristic colour and absorption maximum different from those of the solution given by the new drug.

CALIBRATION CURVE

The colours obtained under the optimum experimental conditions follow Beer's Law over the range of 1 to 25 μg/ml of the drug as base.

RECOVERIES

(A) *From aqueous solutions.* Sodium carbonate solution (2 ml; 1%) and benzene (20 ml) were added to the drug in solution (10 ml) of various concentrations to be tested, such as 1·0, 2·0, 5·0, 10·0 and 20·0 μg/ml, in a separating funnel. The mixture was shaken 300 times/min and after 15 min the benzene layer was removed. The aqueous layer was extracted

twice with benzene, 2 × 15 ml. To the pooled benzene extracts (50 ml) was added 0·002N sulphuric acid (10 ml) and the mixture then shaken for 15 min, the acidic aqueous layer being removed. The ninhydrin reagent (4·0 ml) was added to 2·0 ml of the aqueous solution, the mixture allowed to stand for 24 hr at room temperature to develop the colour, and this was measured in a spectrophotometer. Results in Table 1 show the recovery to be about 97·4 ± 2·1% over the range of 1·0–20·0 μg/ml of drug.

TABLE 1. RECOVERIES OF 3-(DITHIEN-2-YLMETHYLENE)-1-METHYLPIPERIDINE FROM AQUEOUS SOLUTION AND URINE

Aqueous solution				Human urine			
Conc. μg/ml	No. of symples	Found average μg/ml	Found average %	Conc. μg/ml	No. of samples	Found average μg/ml	Found average %
1·0	12	1·00 ± 0·05	100·0 ± 4·9	1·0	5	0·98 ± 0·06	98·2 ± 5·6
2·0	10	1·89 ± 0·16	94·5 ± 8·2	2·0	10	1·96 ± 0·08	98·0 ± 4·0
5·0	13	4·70 ± 0·17	94·0 ± 3·4	5·0	11	4·84 ± 0·31	96·8 ± 6·2
10·0	14	10·1 ± 0·43	100·6 ± 4·3	10·0	7	10·1 ± 0·36	101·1 ± 3·6
20·0	9	19·5 ± 0·90	97·7 ± 4·5	20·0	10	18·9 ± 0·70	94·5 ± 3·5
Average 97·4 ± 2·1%				Average 97·4 ± 1·7%			

(B) *From human urine.* Sodium carbonate solution (2 ml) was added to urine (10 ml) in which 1·0, 2·0, 5·0, 10·0 or 20·0 μg of drug/ml had been dissolved. Each alkaline urine sample was extracted with benzene as described in A. The recovery was about 97·4 ± 1·7% over the range of 1·0–20·0 μg/ml (Table 1).

Acknowledgements. We should like to express our sincere thanks to Dr. N. Sugimoto, Chief of Research Institute of Tanabe Pharmaceutical Co. Ltd., and Dr. R. Kimura, Kyoto University, for their generous supplies of thiophen derivatives used in this experiment, and to Professors Y. Tanaka and T. Murata of this Faculty for their kind advice and encouragement throughout this work.

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