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ASSAY OF 3-SUBSTITUTED PYRIDINES THROUGH FUJIWARA REACTION

M. Atef Abdel-Kader, Aly M. Taha and S. Abdel-Fattah.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, Egypt.

3-substituted pyridines of pharmaceutical interest with a carbonyl group in the 3-position have been determined by a modification of the Koenig's reaction without interferences from the 2-and 4-substituted isomers.

Nicotinic acid, nicotinamide, and niketha-mide in a strongly alkaline medium were reacted with chlorbutol in absolute ethanol on a steam bath for 5 minutes. The reaction mixture was cooled in ice bath and the pH was adjusted. The chromogen was developed by the use of a solution of thiobarbituric acid, barbituric acid or benzidine dihydrochloride where a stable color was formed showing a prominent absorption peaks at 535 , 580 , and 525 nm respectively. The relationship between absorbance and concentration is linear in the range of 0.005 - 0.05 mg per ml for nicotinic acid, and 0.01 - 0.1g per ml for nicotinamide and niketha-mide. The selectivity of the method within the 3-substituted members was also investigated

One of the frequently used colorimetric methods for drugs contanining a pyridine ring is the Koenig's reaction[1,2] .This reaction is based on ring fission by cyanogen bromide followed by condensation of the resulting dialdehyde with various arylamines[3-6], or barbituric and thiobarbituric acids[7,8]. A number of other active halogen compounds have been used to affect ring fission such as cyanogen chloride[7], 2,4-dinitrochloro- or fluorobenzene[9-12], phosphorus trichloride [1], and many others[1].

These reagents vary with regard to ring opening capability for various substituted pyridines. Thus the cyanogen halides are

capable of ring splitting of 3-, 4- and some 2-substituted pyridines , while 2,4-dinitrochlorobenzene does not react at all with the 2-substituted derivatives[2].

Owing to the special handling required for the cyanogen halides, their relative toxicity and practical difficulty in their in situ preparation [1], it was though worthwhile to investigate some other gem-polyhalogenated derivatives as ring openers to investigate their relative selectivity, sensitivity of the method, and practicability of the assay. The present work is aimed at investigating chlorbutol as a reagent for the colorimetric determination of various pyridine drugs.

Experimental

Spectra were made on two spectrophotometers using 1 cm path cells:

1- A single beam spectrophotometer (Spektromom 203, Mom , Budapest, Hungary).

2- Jean and Constant Spectrophotometer(Model 5886, visible range , (Prolabo, Paris).

Materials

Pharmaceutical grade nicotinic acid, nicotinamide and nikethamide have been analyzed as purchased and were used as working standards. Pharmaceutical grade nicotinyl alcohol, methyl nicotinate, α-tocopherol nicotinate, inositol nicotinate, N-hydroxymethylnicotinamide and metyrapone were obtained as gifts from various manufacturers and also used as working standards without further treatment.

Nicotine and other chemicals used were reagent grade. Solvents used were spectrograde or rendered so by the proper treatment[13].

Dosage Forms :

The following commercial preparations were analyzed :

1- Nicotinic acid ampoules (Medimpex); 25 mg per ampoule.

2- Nicyl-papaverine ampoules (Lematle); 40 mg nicotinic acid , and 45 mg papaverine hydrochloride per ampoule.

3- Nicotinamide ampoules (Misr); 50 mg per ampoule.
4- Genosal tablets (Cid); 20 mg nicotinamide, 250 mg sodium gentisate, 250 mg salicylamide, 50 mg vitamin C, and 5 mg vitamin B_1 per tablet .
5- Inhibex tablets (Misr); 5 mg nicotinamide, 50 mg isoniazed, and 5 mg pyridoxine hydrochloride per tablet.
6- Coracid ampoules (Cid); 375 mg nikethamide per ampoule.
7- Coramine-ephedrine ampoules (Ciba); 250 mg nikethamide, and 15 mg ephedrine hydrochloride per ampoule.

Reagents :

1- Chlorbutol solution, 5 per cent w/v in absolute ethanol.
2- Sodium hydroxide solution, 50 per cent w/v in distilled water.
3- Acetic acid solution, 80 per cent w/v of analytical reagent glacial acetic acid in distilled water.
4- Benzidine dihydrochloride, 1 per cent w/v in 0.005 N hydrochloric acid.
5- Thiobarbituric acid, saturated solution in absolute ethanol.
6- Barbituric acid, saturated solution in absolute ethanol.
7- Phosphoric acid (syrupy).

Standard Solutions :

Stock solutions of nicotine, nicotinyl alcohol, 3-aminopyridine, nicotinic acid, benzyl nicotinate, N-hydroxymethylnicotinamide, nicotinamide and nikethamide were prepared using the working standards in concentration of 1 mg per ml in distilled water. Stock solution of inositol nicotinate was prepared in concentration of 1 mg per ml in dilute hydrochloric acid. Stock solution of α-tocopherol nicotinate and metyrapone were prepared in concentration of 1 mg per ml in ethanol.

The standard solutions were prepared by dilution of the stock solutions with distilled water so as to contain 0.005-0.05 mg per ml of nicotinic acid or 0.01 - 0.1 mg of either nicotinamide or nikethamide per ml.

Sample Solutionss :

a) Injections-An aliquot was diluted to give a concentration of 0.005 - 0.05 mg per ml of nicotinic acid or 0.01 - 0.1 mg of either nicotinamide or nikethamide per ml

b) Nicotinamide tablets- An aliquot of one tablet from composite of ten finely powdered tablets was dissolved in distilled water and diluted to the concentration of 0.01-01 mg nicotinamide per ml.

Basic Procedure :

a) Using Thiobarbituric acid as Chromogen Developer :

Two millilitres of the prepared solution was transferred into a 10 ml volumetric flask, 1 ml of chlorbutol solution was added followed by 1 ml of sodium hydroxide solution. The mixture was shaken thoroughly and placed on a boiling water bath for 5 minutes. At the end of heating time, the mixture was cooled in an ice bath and phosphoric acid was added to a pH of about 1.5. One millilitre of thiobarbituric acid solution was added and the reaction mixture was again heated on a boiling water bath for 15 minutes. At the end of heating time the mixture was cooled and diluted to volume with distilled water. The absorbance of this solution was taken at 535 nm against a reagent blank similarly treated .

b) Using Barbituric acid as Chromogen Developer :

The same procedure as described under thiobarbituric acid was followed but instead of heating on a water bath, the chromogen was formed at room temperature by allowing to stand for 15 minutes, after which the absorbance of solution was determined at 580 nm against the proper blank.

c) Using Benzidine as Chromogen Developer :

The same procedure as described under thiobarbituric acid was followed, but instead of adding phosphoric acid, acetic acid solution was added to adjust the pH to about 5 then 2.0 ml of benzidine solution was added and completed to 10 ml with absolute ethanol. The absorbance of the solution was read at 525 nm against blank reagent similarly treated.

RESULTS AND DISCUSSION

A previous investigation from this laboratory[14] has developed a method for the estimation of polychloro compounds via the modified Fujiwara reaction using 3-substituted pyridines. In the present investigation, the inverse mode of this reaction has been utilized for the determination of the 3-substituted pyridines using chlorbutol.

Optimization of Variables :

i) Effect of Concentration of Sodium Hydroxide :

Optimum concentration of sodium hydroxide leading to the maximum intensity of color was found to be 5% w/v final dilution (corresponding to 1 ml of 50% NaOH reagent per 10 ml reaction mixture). Higher concentrations did not cause singificant increase in the color intensity while lower concentrations were obviously insufficient to induce ring opening.

ii) Effect of Reaction Time :

Development of the chromogen was enhanced by heating, where the optimum heating time was found to be 5 minutes . Longer periods resulted in a decrease of color intensity .

With barbituric acid or benzidine, the corresponding chromogen was formed on the cold and color intensities reached maximum within 15 minutes, after which time no further increase was observed. Heating for 15 minutes on a boiling water bath was found to be essential in the case of thiobarbituric acid since the color did not form on the cold. In all cases no significant changes in the readings were observed for at least 10-15 minutes after reaching maximum intensity thus allowing sufficient time for convenient measurament. After this period color intensity was found to decrease slowly .

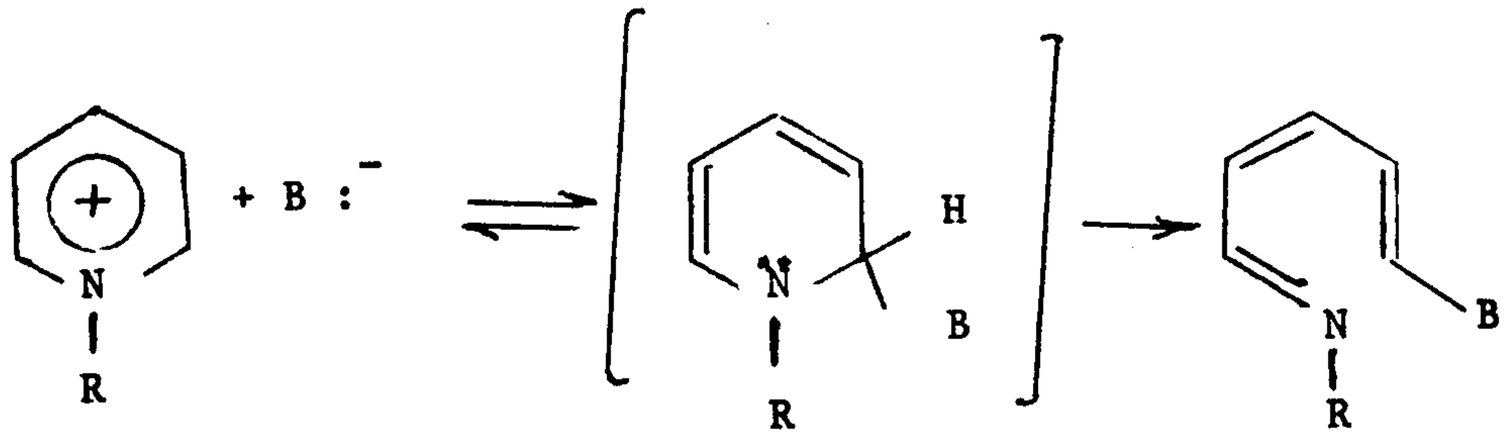
iii) Effect of pH

With thiobarbituric or barbituric acid, the pH was adjusted by the addition of phosphoric acid where the optimum pH was found to be 1.5. On the other hand; pH 5 is recommended for optimum development of color in the case of benzidine. Excess acidity decreased color intensity .

Selectivity :

Different 3-substituted pyridine drugs (Table I), have been examined in order to gain information about the selectivity of the proposed method using thiobarbituric acid. The results suggested an added element of selectivity of chlorbutol reagent as ring-opener. Thus, while cyanogen halides interact with 3-,4-, and some 2-substituted pyridines as well as certain quinolines[1,2] chlorbutol interacted with certain and not all 3-substituted derivatives. The following compounds known to give measurable chromogens with cyanogen bromide[1,2] were tested and gave a negative response : 2-methylpyridine, α, α -dipyridyl, pyridoxine , chloroquine, INH , and chloropheniramine .

Data of Table I suggests that only pyridine drugs substituted at 3-position with substituents having -I-effect (specifically a carbonyl function) give a final chromogen in accord with the following general mechanism :



Thus, any group which acts as an electron acceptor to reduce the electron density of the pyridine ring facilitates attack by the base[1] . Therfore cleavage of the pyridine ring in alkaline medium will be readily achieved by prior quaternization with compounds such as thionyl chloride, chlorosulphonic acid, phosphorus oxychloride, cyanogen halides and others

It may be assumed that quaternization with gem-dihalides does not alone activate the pyridine ring for attack by the base unless a -I substituent is present in the 3-position to enhance susceptibility for ring fission.

Table I - 3-Substituted Pyridine Drugs Subjected to Analysis .

Compound	*Substituent inductive effect*	*Response* ε_{max}	*Response* $A^{1\%}_{1cm}$
Nicotine	+ I	-	-
Nicotinyl alcohol	+ I	-	-
3-Aminopyridine	+ I	-	-
Nicotinic acid	- I	9400	760
Methyl nicotinate	- I	4600	301
Benzyl nicotinate	- I	4000	127
Tocopherol nicotinate	- I	3600	67
Inositol nicotinate	- I	6500	81
Nicotinamide	- I	5700	468
N-Hydroxymethylnicotinamide	- I	1900	125
Nikethamide	- I	11900	670
Metyrapone	- I	3100	137

Accuracy, Precision, and Sensitivity :

Accuracy and precision of the presented method were tested by analyzing aliquots of different batches of standard solutions of nicotinic aicd, nicotinamide, and nikethamide. Absorbance values obeyed Beer's law and calibration curves were found linear with high correlation coefficinets (Table II).

Table II- Results of Analysis by Two Chromogen Developers .

Compound	Mean*	S.D.%	r	Chromogen Developer
Nicotinic Acide	100.93	2.26	0.988	A
	100.75	2.61	0.940	B
Nicotinamide	100.50	1,06	0.997	A
	101.17	1.95	0.960	B
Nikethamide	100.95	2.23	0.990	A
	100.58	0.96	0.990	B

* Average value of at least five determinations.

A: Using thiobarbituric acid as chromogen developer

B: Using benzidine as chromogen developer

Application to Dosage Forms:

The applicability of the method to commercial dosage forms was checked by analyzing seven different relatively simple preparations representing nicotinic acid, nicotinamide, and nikethamide. The amount of the substrate in the preparation was calculated by reference to corrosponding calibration curve.

REFERENCES

1) Pesez M. and Bartos J. "Colorimetric and Fluorometric analysis of Organic Comounds and Drugs", Marcel Dekker, Now York, N.Y. 1974, pp. 330-3.

2) Kakac B. and Vejdolek Z.J. "Handbuch der photometrischen Analyse organischer Verbindungen" B.2, Verlag Chemie, Weinheim, 1974, pp. 771-80.

3) Jones W.S., J. Am, Pharm. Assoc., 30, 272 (1941).

4) Sweeney I.P., and Hall W.L., Anal. Chem., 23, 983 (1951).

5) Shah R.O., Raman P.V., and Gandhi S.D., Indian J. Pharm., 20, 105 (1958).

6) Pelletier O., J. Assoc. Offic. Anal. Chem., 51, 828 (1969).

7) Asmus E. and Garschagen, J. Anal. Chem., 139 , 81 (1953).

8) Mensard P., Devawx G. and Fauquet J., Sci. Pharm. Proc., 25th 2 , 77 (1965), through Ref. (1) p. 602.

9) Karrer P. and Keller H., Helv. Chim. Acta, 21, 463, 1170(1938).

10) Vilter S.P., Spies T. D., and Mathews A. P. Biol. chem., 125 , 85 (1938).

11) Hrington E.F.G., Analyst, 76, 90 (1951).

12) Ballard C.W., and Scott P. G. W. , Chem Ind. (London), 715 (1952); Scott P. G. W. J. Pharm. Pharmacol., 4 , 681 (1952).

13) Reddick J.A. , and Bunger W. B. in " Organic Solvents" 3rd ed. Wiley-Interscience, New York, 1970.

14) El-Kommos M.E., M.S. Thesis, Faculty of Pharmacy. University of Assiut, Egypt, 1974 .

تحليل مركبات البيريدين المحتلة فى الموضع (بيتا)
باستخدام تفاعـــــل فوجيـــــوارا

محمود عاطف عبدالقادر ـ علـى محمود طـــه ســـلام عبدالفتاح
قســم الكيمياء الصيدليـة ـ كليـــة الصيدلـة جامعة اسيوط

تنـاول هـذا البحـث تحليـل مركبـات البيريـدين غير الرباعيـة وذلك بطريقـة دقيقـة دالـة الثبـات.

وتعتمـد هـذه الطريقـة على تكـوين الـمواد الناتجـة من تفاعـل مركبـات البيـريدين مع كلوريتـول ثم تكثيف النـاتج مع حمض البار بتوريك الاكـجينى وكـذلك الكبريـتى او البـنزيدين كجواهرملونه.

ولقـد تمت دراسـة تاثير متغيرات التفاعـل المختلفة مثل تركيز بيريدين وكميـة الصـوديوم ومـدة التسـخين ودرجـة الاس الايدروجينـى وغـيرها على معدل تكـوين اللـون ودرجـة شـدته النهائيـة

وقـد تم استخدام هـذه الطـريقة فى تحليـل مركبـات البيريـدين غير الرباعيـة وعـلى الاخص المركبات المحتـلة فى الموضع (بيتـا) كما استخدمت فى التحليـل الدقيـق لحامـض النيكوتينيك ،نيكوتينـاميد ونيكيتاميـد فى المستحـضرات التجـارية دون الحاجة الى فصـل مسـبق ودون تداخـل.