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Адрес на редакицията
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ул.. "Дунав" 2, София 1000
Факс (02) 987 987 4
Гл. редактор: ✆ (02) 987 987 4
E-mail:snikolov@mbox.pharmfac.acad.bg

Address of Editorial Board
Faculty of Pharmacy
2, Dunav str., Sofia 1000
Fax (02) 987 987 4
Editor in Chief: ✆(+359 2) 987 987 4
E-mail:snikolov@mbox.pharmfac.acad.bg

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ISOLATION AND CHARACTERIZATION OF ISOMERS OF PYRROLE-HYDRAZONES WITH POSSIBLE TUBERCULOSTATIC ACTIVITY. COMPARISON OF METHODS FOR SEPARATION

M. Georgieva[1], A. Bijev[2] and I. Nencheva[2]

[1]Faculty of Pharmacy, Medical University – Sofia

[2]Department "Organic Synthesis and Fuels", University of Chemical Technology and Metallurgy – Sofia

Summary. Hydrazones are a big class of organic compounds, possessing diverse biological activity. Due to the presence of a double bond in their structure, geometrical isomerism is very characteristic for them. Development of a suitable method for separation of geometric isomers thereof will be of essence for future investigations. Two methods for separation of the geometrical isomers of a previously reported hydrazone **11o** were compared: preparative TLC chromatography and fractional re-crystallization, taking into account their simplicity and expeditiousness. TLC was performed on 2 mm Silica gel/Glass plaque. In order to assure better separation, a gradient elution was applied. Both isomeric forms of hydrazone **11o** were successfully separated by TLC preparative chromatography and further characterized and proved by IR and ^{1}H-NMR spectral analysis. The attempt to isolate the isomers of hydrazone **11o** by the second method was unsuccessful. Thus more effective and more applicable appears to be the separation with preparative TLC chromatography.

Key words: geometrical isomerism, pyrrole hydrazones, tuberculostatics

ИЗОЛИРАНЕ И ХАРАКТЕРИЗИРАНЕ НА ПИРОЛОВИ ХИДРАЗОНИ С ЕВЕНТУАЛНА ТУБЕРКУЛОСТАТИЧНА АКТИВНОСТ. СРАВНЯВАНЕ НА МЕТОДИ ЗА СЕПАРИРАНЕ

М. Георгиева[1], А. Бижев[2] и И. Ненчева[3]

[1]Фармацевтичен факултет, Медицински университет – София

[2]Катедра „Органичен иснтез и горива", Химикотехнологичен и металургичен университет – София

Резюме. Хидразоните са голям клас органични съединения, притежаващи разнообразна биологична активност. Поради наличието на двойна връзка в структурата им характерно за тях е наличието на геометрична изомерия. Разработването на подходящ метод за разделяне на геометричните им изомери е от съществено значение за бъдещи разработки. Бяха сравнени два метода за разделяне на изомери на вече публикувания в предишни разработки хидразон **11o**: препаративна ТСХ хроматография и фракционна прекристализация, предвид тяхната простота на изпълнението и бързина. ТСХ бе проведена на 2 mm Silica gel/стъклена плака. С цел постигане на по-добро разделяне бе приложено градиентно елуиране. Успешно бяха разделени двете изомерни форми на хидразон **11o**, които бяха охарактеризирани и доказани с помощта на IR и 1H-NMR спектрален анализ. Опитът за изолиране на изомерите на хидразон **11o** чрез втория метод се оказа неуспешен. Така методът на препаративна ТСХ хроматография се оказа по-приложим и по-ефективен при разделянето на изомерите на разглеждания хидразон.

Key words: геометрична изомерия, пиролови хидразони, туберлкулостатичност

Introduction

Hydrazones, containing a pyrrole cycle in their structure, are a big class of organic pyrrole compounds, arousing great interest lately. The biological activity of this class compounds is of great variety. Hydrazones are famous for their antidepressant, analgesic, anti-malarial, anti-tubercular, anti-viral etc. activity [4]. Besides the various activity, hydrazones are known to exhibit geometrical isomerism, assigned to the presence in their structure of the

C=N double bond. As a result for a number of hydrazone compounds, a relationship between the corresponding isomeric form and the biological activity has been established.

The geometrical isomerism is of great importance for the biological activity expression of the compounds, since it is essential for the interaction of the drug molecule with the receptor. The following dependence of the biological activity on the geometrical isomerism is observed: both isomers may possess equal activity; only one of the isomers may be biologically active; the two isomeric forms may express controversial or different pharmacological effect; one of the isomers may display undesired side activity; etc.

In an attempt to isolate and identify the possible isomeric forms of the compounds, a number of techniques and methods has been analyzed. From the so performed experiments has been concluded that the chromatographic techniques have been the most useful and most widely applied for analysis of isomeric purity [13]. These methods offer distinct advantages, including small sample size, independence from the magnitude of specific rotation, and independence from other species, initially present. Research in recent years has produced a number of significant advances in chromatographic separation techniques for isolation of isomers [5, 8, 14].

In most cases, geometric isomers posses close physicochemical properties, which perplex their separation. Sometimes, some differences in the solubility of the both isomeric forms are observed. In this case, for their separation a fractional re-crystallization may be applied. In this method, a mixture of both isomers is dissolved in appropriate solvent and let to crystallize at ambient temperature. As a result, the less soluble isomeric from will precipitate first. After consecutive concentration of the solution, a second crystal portion is separated – the second isomeric from [7].

The aim of the current study is to investigate and establish suitable method for separation of geometric isomers of pyrrole-containing hydrazones. For this purpose, two methods are applied for separation of the isomers of a model hydrazone: a preparative TLC chromatography and fractional re-crystallization, due to their simplicity and expeditiousness.

As a model compound, pyrrole hydrazone **11o** [2] was chosen, considering its high biological activity and the presence of two chromatographic spots with close Rf values in the corresponding TLC chromatogram of the obtained product and the observed splitting in the signals of the respective ^{1}H-NMR spectra.

Methods

Apparatuses and devices

For determination of the melting points, a capillary Digital Melting Point Apparatus IA 9200 ELECTROTHERMAL (Southend-on-Sea, England) was used and the obtained values were not corrected. The FT-IR spectra were recorded on a Varian Scimitar 1000 Spectrometer in KBr. The ^{1}H-NMR spectra were registered at 250 MHz on spectrometer Bruker-Spectrospin WM250MHz (Faenlanden, Switzerland) as δ (ppm) relative to TMS as internal standard and the coupling constants (*J*) are expressed in Hertz (Hz). All OH and NH protons were D_2O exchangeable.

Isolation of the geometric isomers

Using preparative TLC chromatography technique

For performance of the preparative Thin Layer Chromatography (TLC) separation, a 2 mm Silica gel/Glass plaque was used as stationary phase. A mobile phase with contiguous increase in the polarity was applied, in order to achieve gradient elution using the following series of mobile phases:

1) Benzene:methanol:acetonytril:tetrahydrofurane =10:0,2:0,2:0,5;

2) Benzene:methanol:acetonytril:tetrahydrofurane =10:0,3:0,3:0,7;

3) Benzene:methanol:acetonytril:tetrahydrofurane =10:0,5:0,3:0,7.

Using fractional re-crystallization method

In the following study, to a previously determined quantity of hydrazone ethyl 5-(4-bromophenyl)-2-methyl-1-(2-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)ethyl)-1H-pyrrole-3-carboxylate (**11o**), 10 ml of ethanol was added and the obtained mixture was heated on water bath, until full clarification of the solution. Then the solution was let to crystallize at ambient temperature. As a result a less soluble product crystallized and thus the first portion of crystals was isolated. After its re-crystallization from a pure solvent, the desired product was obtained and further elucidated by IR and ^{1}H-NMR spectral analyses. After consecutive concentration of the solution, the second crystal portion was separated – the second product. Thus two products were separated and isolated from the mixture.

Results and Discussion

The synthesized by us in previous investigations compound **11o** (fig. 1) [2] is colored crystals

insoluble in water and a number of organic solvents. Well soluble in dimethylsulfoxide (DMSO) and tetrahydrofurane (THF).

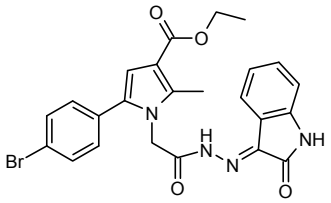

Fig. 1. *General formulae of hydrazone* ***11o***

The structure of the obtained from the synthesis product was confirmed by IR and ^{1}H-NMR spectral analyses. On the corresponding ^{1}H-NMR spectra, some splitting in the signals is observed, whereat one of the peaks is predominant (Fig. 2).

This let us to believe, that is due to the presence of two geometrical isomeric forms.

Separation using TLC technique

The TLC has been extensively applied to geometrical isomers separation, since the mechanism of separation and the structure-selectivity relationship is best studied for this technique [1, 10].

In the present study, a preparative TLC method was developed for separation of the two isomeric forms of previously synthesized and reported hydrazone ethyl 5-(4-bromophenyl)-2-methyl-1-(2-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)ethyl)-1H-pyrrole-3-carboxylate (**11o**). Number of chromatographic conditions were analyzed and evaluated as possible TLC systems for separation of the E/Z forms. Of all 21 experimented two-, three- and four-component chromatographic systems, most appropriate was established to be the following mobile phase:

Benzene:methanol:acetonytril:tetrahydrofurane =10:0,5:0,5:1,25;

This mobile phase and 0.2 mm Silica gel/ alluminium sheet plaque used as stationary phase was chosen for the preliminary development of a suitable TLC chromatographic system. Using the above selected chromatographic conditions, two spots were observed with corresponding R_f – values different from the values of the initial reagents, whereas for the selected hydrazone **11o** the both values are as follows: R_{f1} = 0,1 and R_{f2} = 0,43. This makes the selected system suitable as a base for further attempts to separate the both isomers of the chosen compound.

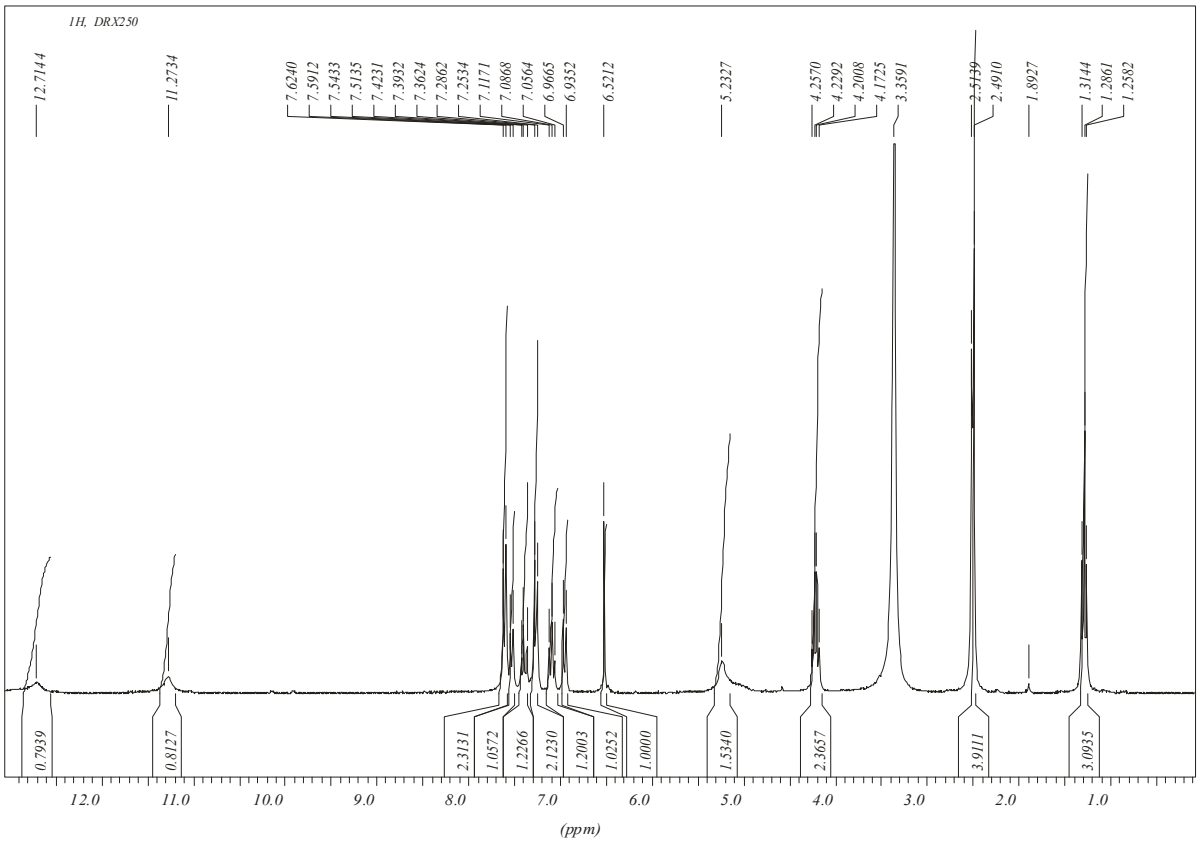

Fig. 2. ^{1}*H-NMR of* ***11o***

In order to obtain quantities, sufficient for further structure elucidation, the separation was performed on 2 mm Silica gel/Glass plaque in a preparative TLC scale format. Some overlapping in the layers was observed in the above cited system, which forced us to change conditions from isocratic to gradient elution. The latter was achieved by contiguous increase in the polarity of the mobile phase, obtained by consecutive elution in the above described conditions. Thus separation of two products was achieved. The structure of the obtained products was elucidated and proven by corresponding melting points and IR- and ^{1}H-NMR-spectral analyses.

It is well known that for the predominant isomeric form the formation of stabilizing intermolecular hydrogen bonds is of essential importance [9, 11]. On the other hand, the isomeric form with lower internal energy would express higher affinity to the solvent and would be eluted first [1]. Thus it is obvious that the isomer able to form hydrogen bonds would be eluted latter, and would posses lower R_f-value.

For the analysed compound, the corresponding E/Z-form was assigned to the structure, according to Cahn–Ingold–Prelog priority rules, whereat when the hydrogen atom of the NH group from the hydrazide part of the molecule and the free carbonyl group from the Isatine are in the same side of the C = N double bond, the arrangement is Z, if they are on opposite sides, the arrangement is *E*.

In the chosen structure, the Z-isomeric form seems to be more stable due to its ability to form intermolecular hydrogen bond, between the free carbonyl group from the Izatine part of the molecule and the proton of the CONH group of the amide part of the structure (Fig. 3). Thus as an additional stabilization of the molecule, a six member cycle is formed also.

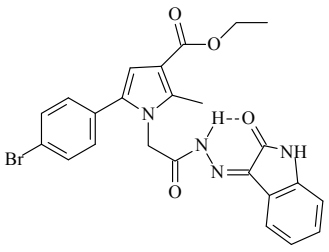

Fig. 3**. Structure of the Z-isomeric form of hydrazone **11o

In the other case (the E-isomeric form), the formation of this type of hydrogen bond is impossible, due to the spatial arrangement of the carbonyl group from the carbonyl part of the hydrazone, and the corresponding proton from the CONH group (Fig. 4).

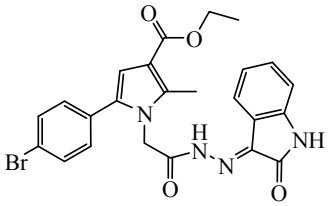

Fig. 4.** Structure of the E-isomeric form of hydrazone **11o

An additional conformation of this is the difference in the melting points of the isolated products, where the m.p. for the Z-isomeric form is lower, than the m.p. for the E-isomer: **11o/Z** = 246°C and **11o/E** = 269°C respectively.

This fact was also confirmed by the performed IR spectral analyses. In the corresponding IR spectra, taken in KBr (cm^{-1}) the following bands are observed: **11o/E**: 3270 with shoulder at 3250 (ν NH), 2927 (ν CH_3, CH_2), 1693 with shoulder at 1687 (ν C=O, -COOC2H5, ν C=O Izatin), 1621 (ν C=O (Amide I)), 1564 (δ NH (Amide II)), 1463 (δ CH_2), 1379 (δ CH_3), 1248 (ν C-O, -$COOC_2H_5$), 831 (p-substituted benzene ring) and for **11o/Z**: 3265 (ν NH), 2977 (ν CH_3, CH_2), 1690 with shoulder at 1678 (ν C=O, -$COOC_2H_5$, ν C=O Izatin), 1620 (ν C=O (Amide I)), 1570 (δ NH (Amide II)), 1523 (ν C=N), 1463 (δ CH_2), 1378 (δ CH_3), 1247 (ν C-O, -$COOC_2H_5$), 831 (p-substituted benzene ring) respectively.

The observed low frequency of the carbonyl groups may be a result of the intramolecular H-bonding. The low stretching vibrations also observed for the ester carbonyl group may be due to the hydrogen bonding with the pyrrole CH3 group [3].

It is worth mentioning that the spectra of compound **11o/E** exhibit a shoulder in the spectrum at 1687 cm^{-1}. This band can be assigned to the stretching mode for the free C=O group of Isatine. An expected transposition of thus formed shoulder to lower values (1678 cm^{-1}) is observed in the spectra of **11o/Z**. This is due to the contribution of the C=O group to hydrogen bonding with the proton of the NH group of the hydrazide part of the molecule [6]. Analogical downshift to the lower values of the stretching vibrations is observed for the Amide II groups. It is worth mentioning also, that the area between 3100 and 3300 cm^{-1} is wide and complex and a general decrease in the intensity is observed, as compared to the spectrum of **11o/E**.

This is confirmed by the further made ^{1}H-NMR analysis. In the obtained spectra, the proton signals assigned to the E- form CO<u>NH</u>, CH_2<u>CH_3</u>, <u>CH_3</u> (2)

and Isatin-NH groups are downfield, as compared to the signals of the Z- form. The downfield shift of these signals supports the contribution of the formed intramolecular H– bond [6, 12]. The percentages of E- and Z-conformers are calculated from the ratio of the integral intensities of these signals. It was found that the more stable Z-isomeric form dominates.

The proton shifts in the ^{1}H-NMR spectra, assigned to the corresponding groups are presented on the following Table 1.

Separation by fractional re-crystallization

An attempt was made to separate the two isomeric E-/Z- forms of the reported hydrazone **11o** by fractional re-crystallization method. Due to the very similar physico-chemical properties of both isomers, only one product was obtained in quantities sufficient for its structural elucidation. The structure of the isolated product was elucidated with melting point determination and IR- and ^{1}H-NMR – spectral data. The result from the performed structure elucidation analysis showed, that the obtained structure is the one for the predominant isomeric form **11o/Z** with: m.p. 246°C; **IR (KBr) cm^{-1}**: 3265 (ν NH), 2977 (ν CH_3, CH_2), 1690 with shoulder at 1678 (ν C=O, -$COOC_2H_5$, ν C=O Izatin), 1620 (ν C=O (Amide I)), 1570 (δ NH (Amide II)), 1523 (ν C=N), 1463 (δ CH_2), 1378 (δ CH_3), 1247 (ν C-O, -$COOC_2H_5$), 831 (p-substituted benzene ring) and corresponding **^{1}H-NMR (DMSO-d$_6$) δ, ppm:** 1,28 (t, 3H, J=7,1, CH_3CH_2), 2,45 (s, 3H, CH_3(2)), 4,16 (q, 1H, J=7,14, CH_3CH_2), 4,22 (q, 1H, J=7,1, CH_3CH_2), 5,10 (s, 2H, CH_2CO), 6,53 (s, 1H, H(4)), 6,83 (d, J=7,8, Isatin-C_6H_4-7), 6,96 (t, Isatin-C_6H_4-6), 7,11 (d, J=8,32, C_6H_4-3,5), 7,27 (t, Isatin-C_6H_4-5), 7,39 (t, d, J=8,31, C_6H_4-2,6), 7,27 (t, Isatin-C_6H_4-8), 10,44 (s, 1H, Isatin-NH), 10,87 (s, 1H, CONH).

It was established, that by this method we have achieved a good purification of the predominant and more stable Z-isomeric form. The second product (the corresponding E-isomeric form) was obtained only in traces, and its purification and further elucidation at this point was unsuccessful.

Conclusion

A preparative TLC chromatographic system was applied for separation of the isomeric forms of preciously synthesized hydrazone **11o**. The isomers of the chosen compound were successfully separated and their structure was elucidated by melting points, IR and ^{1}H-NMR analysis. Both isomers were proved to be free of any impurities detectable by TLC analysis. The Z-isomer exhibited lower R_f value, than the corresponding E-isomer in normal phase system. The percentages of E- and Z-isomers were calculated from the ratio of the integral intensities of these signals. It was found that, the more stable Z-isomeric form dominates. A fractional re-crystallization method was also applied in an attempt to isolate the two isomeric forms of the hydrazone of interest. It was determined, that this method is not suitable, since no separation was achieved. Establishment of the biological activity of the obtained purified isomeric forms and determination of the isolated new product are forthcoming.

Table 1. *Chemical shifts (δ/ppm) from ^{1}H-NMR spectra (DMSO-d6) of E-/Z-isomers of hydrazone 11o.*

Hydrazone	CH_2CH_3 t	CH_3(2) s	Pyr-H s	Isatin-NH s	CONH s	Other chemical shifts
E-	1.35	2.53	6.59	10.92	12.63	4.22(q, J=7.09 Hz, CH_2CH_3) 4.30(q, J=6.95 Hz, CH_2CH_3) 5.13(s, N-CH_2CO) 6.90(d, J=7.5 Hz, Isatin-C_6H_4-7) 7.04(t, Isatin-C_6H_4-6) 7.20(d, J=7.5 Hz, C_6H_4-3,5) 7.32(t, Isatin-C_6H_4-5) 7.49(d, J=8.07 Hz, C_6H_4-2,6) 7.62(t, Isatin-C_6H_4-8)
Z-	1.28	2.45	6.53	10.44	10.87	4.16(q, J=7.14 Hz, CH_2CH_3) 4.22(q, J=7.1 Hz, CH_2CH_3) 5.1(s, N-CH_2CO) 6.83(d, J=7.8 Hz, Isatin-C_6H_4-7) 6.96(t, Isatin-C_6H_4-6) 7.11(d, J=8.32 Hz, C_6H_4-3,5) 7.27(t, Isatin-C_6H_4-5) 7.39(d, J=8.31 Hz, C_6H_4-2,6) 7.44(t, Isatin-C_6H_4-8)

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✉ **Address for correspondence:**
Maya Georgieva
Faculty of Pharmacy
Medical University
2, Dunav Str.
1000 Sofia
✆ +359 2 9236515;
e-mail address: georgm@mail.bg

✉ **Адрес за кореспонденция:**
Мая Георгиева
Фармацевтичен факултет
Медицински университет
ул. "Дунав" №2
1000 София
✆ +359 2 9236515;
e-mail: georgm@mail.bg