REACTIVITY OF PHENYLANTHRANILIC ACIDS DERIVATIVES. XXIII. SYNTHESIS AND ACID-BASE PROPERTIES OF 4,5-DIHYDROXY-N-(2'-CARBOXYPHENYL)ANTHRANILIC ACIDS

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Key words: synthesis; reactivity; N-phenylanthranilic acid

The alternative ways of synthesis of new 4,5-dihydroxy-N-(2'-carboxyphenyl)anthranilic acids have been considered and new ways for their obtaining have been suggested. The structure of the compounds synthesized has been proven by the elemental analysis, IR- and NMR-spectroscopy. The purity has been controlled by the method of thin-layer chromatography. The reactivity of 4,5-dihydroxy-N-(2'-carboxyphenyl)anthranilic acids has been researched by studying the acid-base properties in the binary solvent of dioxane-water (60 vol% of dioxane). It has been found that the substances synthesized are dibasic subacids, which strength depends upon the nature and position of substituents. The quantitative assessment of the influence of substituents on two reactive centres of the acids synthesized has been carried out by the method of correlative analysis according to the Gamete equation. It has been proven that the reactive centres sensitivity is substantially different and dependent on the substituent distance. In addition, appearance of another reactive centre does not practically influence on sensitivity of the first one. It has been determined that the substances synthesized reveal the anti-inflammatory, analgesic, diuretic, bacteriostatic, and fungistatic effects. According to the classification by K.K. Sydorov the substances synthesized when introducing intragastrically belong to low-toxic compounds (DL50 > 3000 mg/kg).

РЕАКЦИОННАЯ СПОСОБНОСТЬ ПРОИЗВОДНЫХ N-ФЕНИЛАНТРАНІЛОВИХ КИСЛОТ. XXIII. СИНТЕЗ ТА КИСЛОТНО-ОСНОВНІ ВЛАСТИВОСТІ 4,5-ДИМЕТОКСИ-N-(2'-КАРБОКСИФЕНИЛ)АНТРАНІЛОВИХ КИСЛОТ

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Ключові слова: синтез; реактивність; N-фенілантраціловая кислота

Розглянути альтернативні варіанти синтезу нових 4,5-диметокси-N-(2'-карбоксифеніл)антрацілових кислот та запропоновано нові спосібі їх одержання. Будь-яку синтезовані речовини доведено даними елементного аналізу, ІХ- та ПМР-спектроскопії. Чистоту контролювали методом тонкослійної хроматографії. Досліджено реакцію стійкості заміщенних N-(2'-карбоксифеніл)антрацілових кислот шляхом вивчення кислотно-основних властивостей у бінарному розчині діоксан-вода (60 об% діоксуану). Встановлено, що синтезовані речовини є слабкими двостатковими кислотами, сила яких залежить від природи та положення замісників. Методом кореляційного аналізу проведено кількісну оцінку впливу замісників на два реакційні центри синтезованих кислот за рівнянням Гаммента. Доведено, що чутливість реакційних центрів суттєво відрізняється та залежить від віддаленості замісників. При цьому появу другого реакційного центра не впливала на чутливість первинного. Було встановлено, що синтезовані речовини проявляють протизапальну, аналгетичну, діуретичну, бактеріостатичну та фунгістатичну активність. За класифікацією К.К.Сидорова синтезовані речовини при внутрішньошлюковому введенні належать до класу малотоксичних сполук (ДЛ50>3000 мг/кг).

РЕАКЦИОННАЯ СПОСОБНОСТЬ ПРОИЗВОДНЫХ N-ФЕНИЛАНТРАНІЛОВИХ КИСЛОТ. XXIII. СИНТЕЗ И КИСЛОТНО-ОСНОВНЫЕ СВОЙСТВА 4,5-ДИМЕТОКСИ-N-(2'-КАРБОКСИФЕНИЛ)АНТРАНИЛОВЫХ КИСЛОТ

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Ключевые слова: синтез; реактивность; N-фенилантираноловая кислота

Рассмотрены альтернативные варианты синтеза новых 4,5-диметокси-N-(2'-карбоксифенил)антраниловых кислот и предложены новые способы их получения. Строение синтезированных веществ доказано данными элементного анализа, ИК- и ПМР-спектроскопии. Чистоту контролировали методом тонкослойной хроматографии. Исследована реакционная способность замещенных N-(2'-карбоксифенил)антраниловых кислот путем изучения кислотно-основных свойств в бинарном растворителе дioxан-вода (60 об% дioxана). Установлено, что синтезированные вещества являются слабыми двуосновными кислотами, сила которых зависит от природы и положения заместителей. Методом корреляционного анализа проведено количественную оценку влияния заместителей на два реакционных центра синтезированных кислот согласно уравнению Гаммета. Доказано, что чувствительность реакционных центров существенно отличается и зависит от удалённости заместителей. При этом появление второго реакционного центра практически не влияет на чувствительность первого. Было установлено, что синтезированные вещества проявляют противовоспалительную, анальгетическую, диуретическую, бактериостатическую и фунгистатическую активность. По классификации К.К.Сидорова синтезированные вещества при внутривенном введении относятся к классу малотоксичных соединений (ДЛ50>3000 мг/кг).
Analysis of the research data [1-11] indicates the widespread use of derivatives of N-phenylanthranilic acids in medicine, pharmacy, industry and various fields of science. N-phenylanthranilic acids derivatives have a wide synthetic and pharmacological potential [1-3, 8-11]. The circumstances above caused the necessity to carry out the synthesis of new 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids, study of their reactivity and biological activity. It will allow to optimize the search for new biologically active compounds of this series and forecast their biological effect.

Substituted 4,5-dimethoxy-N-(2’-carboxyphenyl) anthranilic acids have been obtained by the Ullmann reaction by the interaction of 4,5-dimethoxy-chlorobenzoic acids (1) with o-halogenbenzoic acid (method 1) and by arylation of 4,5-dimethoxoanthranilic acid by o-halogenbenzoic acid (method 2) in the medium of N-amylalcohol (method 1A, 2A), DMF (method 1B, 2B), without a solvent (method 1C, 2C) in the presence of copper or copper (II) oxide [1-3]. The same as a counter synthesis of 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids (5) condensation of N-acetyl-4,5-dimethoxyanthranilic acids (3) with substituted o-halogenbenzene acid follow by hydrolysis of N-acyd derivatives has been used (Method 3). In order to increase solubility of copper ions in the aprotic low-polar phase to accelerate the arylation reaction various solvent (Tween-80, sodium salt of oleic acids, stearic acid) added to the reaction mixture in the amount of 3-5 weight percent have been used. The use of sodium oleate as a phase transfer catalyst allows to accelerate 1,4 times the arylation reaction (Scheme 1).

The structure and identity of 4,5-dimethoxy-N-(2’-carboxyphenyl) anthranilic acids have been confirmed by elemental analysis, IR-, NMR-spectroscopy, chromatographic analysis and qualitative reactions (Table 1, 2, 3).

IR-spectra of 4,5-dimethoxy-N-(2’-carboxyphenyl) anthranilic acids (5a-f) are characterized by a number of intense bands, which correspond to the main structural fragments of molecules of the substances synthesized, \( \nu, \text{cm}^{-1}: 3362-3258 (\nu_{\text{NH}}), 1709-1659 (\nu_{\text{C}=\text{O}}), 1591-1570 (\delta_{\text{NH}}), 1235-1206 (\nu_{\text{CN}}) \). The strong bands of nitrogroup \( \nu_{\text{NO}}^\text{R} \), 1523 \text{cm}^{-1} and \( \nu_{\text{NO}}^\text{R} \), 1351-1350 cm\(^{-1}\) are also characteristic for the spectra of compounds (5a-f).

In the NMR-spectra of acids (5a-f) signals of aromatic protons in the range of 6.50-7.90 ppm have been identified. The proton signals of the secondary amino group appear as a broad singlet in the region of 8.05-11.18 ppm. The proton signals of the methoxy group are one or two singlets at 3.60-3.80 ppm (Table 3).

As a continuation of a number of papers [4, 12-19] related to the study of reactivity of biologically active substituted N-phenylanthranilic acids, dissociation of 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids (5a-f) has been examined according to the equation.

Ionization constants of 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids have been determined by potentiometric titration in the binary solvent of dioxane-water (60 vol\% of dioxane) at 25°C (Scheme 2) [18, 19]. The given method allows to obtain concentr-
### Table 1

Characteristics of 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids synthesized

| Compound | R     | Yield, % | M.p., °C | Found, % | Formula | Calculated, % |
|----------|-------|----------|----------|----------|---------|--------------|
|          |       | Method 1 | Method 2 | Method 3 |         |              |
|          | A     | B        | C        | A        | B       |              |
| 5a       | H     | 62       | 69       | 88       | 63      | 74           | 88           | 35        | 50     | >300   | 4.45  | 60.61 | C_{16}H_{14}NO_{6} | 4.41 | 60.57 |
| 5b       | 4´-Br | 67       | 70       | 92       | 65      | 74           | 90           | 45        | 56     | >300   | 3.59  | 48.53 | C_{16}H_{14}BrNO_{6} | 3.54 | 48.51 |
| 5c       | 5´-Cl | 68       | 71       | 90       | 65      | 72           | 89           | 47        | 55     | 260-264 | 3.91  | 54.58 | C_{16}H_{14}ClNO_{6} | 3.98 | 54.64 |
| 5d       | 6´-NO₂ | 65      | 74       | 92       | 62      | 70           | 90           | 44        | 57     | >300   | 7.82  | 52.99 | C_{16}H_{14}NO_{6} | 7.73 | 52.99 |
| 5e       | 4’,6´-Br | 65     | 75       | 90       | 63      | 72           | 88           | 40        | 55     | >300   | 3.05  | 40.41 | C_{16}H_{14}BrNO_{6} | 2.95 | 40.45 |
| 5f       | 4’,6´-NO₂ | 69     | 79       | 94       | 65      | 75           | 93           | 43        | 55     | 254-257 | 10.38 | 47.22 | C_{16}H_{14}N_{2}O_{8} | 10.32 | 47.18 |

Note. 1 Crystalized from aqueous ethanol

### Table 2

Acid-base, spectral and chromatographic characteristics of 4,5-dimethoxy-N-(2’-carboxyphenyl)anthranilic acids

| Compound | R     | pKa(1)  | pKa(2)  | NMR-spectra, chemical shifts (ppm) | IR-spectra, absorption maxima, cm⁻¹ | R_{f}¹ |
|----------|-------|---------|---------|-----------------------------------|-------------------------------------|-------|
|          |       |         |         | COOH (1H, s.) NH (1H, w.s.) OCH₃ ArH | v_{NH} v_{C=O} v_{CPh} δ_{NH} ν_{as}stretch NO₂ ν_{CNH} |       |
| 5a       | H     | 6.37±0.02 | 6.95±0.03 | 12.95 10.65 3.71 (6H,s) 6.80-7.99(6H,m) | 3350 1659 1597 1575 - 1235 0.45 |       |
| 5b       | 4´-Br | 5.74±0.02 | 6.72±0.02 | 13.01 10.70 3.65 (6H,s) 6.88-7.90(5H,m) | 3356 1668 1613 1582 - 1213 0.40 |       |
| 5c       | 5´-Cl | 6.14±0.01 | 6.74±0.03 | 12.93 10.72 3.75 (6H,s) 6.69-7.80(5H,m) | 3362 1669 1613 1583 - 1234 0.43 |       |
| 5d       | 6´-NO₂ | 5.14±0.03 | 6.55±0.01 | 13.10 10.92 3.82 (6H,s) 7.05-8.75(5H,m) | 3258 1675 1616 1589 1570 1523 1351 1206 0.46 |       |
| 5e       | 4’,6´-Br | 3.75±0.01 | 5.76±0.02 | 12.98 8.05 3.72 (3H,s) 3.60(3H,s) 6.50-7.33(4H,m) | 3348 1665 1602 1576 - 1230 0.35 |       |
| 5f       | 4’,6´-NO₂ | 3.2±0.03  | 5.83±0.01 | 12.90 10.80 3.80 (6H,s) 6.72-7.55(4H,m) | 3332 1709 1672 1614 1591 1523 1350 1211 0.38 |       |

Note. ¹R_{f} values are given in the solvent system of ethanol-hexane (1:2.5)
The proton chemical shift value of 4,5-dymethoxy-N-(2′-carboxyphenyl)anthranilic acids

| Compound | R   | COOH (1H, s) | NH (1H, w.s) | (OCH₃)₃ | ArH                  |
|----------|-----|--------------|--------------|---------|---------------------|
| 5a       | H   | 12.95        | 10.65        | 3.71    | 6.80-7.85 (6H, m)   |
| 5b       | 4′-Br | 12.93       | 10.72        | 3.75 (6H, c) | 6.69-7.80 (5H, m) |
| 5c       | 5′-Cl | 12.90        | 10.80        | 3.80 (6H, s) | 6.72-7.55 (4H, m) |
| 5d       | 6′-NO₂ | 13.01        | 10.70        | 3.65 (6H, s) | 6.88-7.90 (5H, m) |
| 5e       | 4′,6′-Br | 12.98       | 8.05         | 3.72 (3H, s) | 6.50-7.30 (5H, m) |
| 5f       | 4′,6′-NO₂ | 13.20       | 11.18        | 3.80 (6H, s) | 7.10-8.70 (5H, m) |

The data presented in Table 2 testify that the nature and position of substituents have influence upon the acid-base balance of both 4,5-dymethoxy-N-(2′-carboxyphenyl)anthranilic acids. Introduction of electron-acceptor substituents strengthens dissociation of acids due to the greater anion stabilization. Electron donor substituents cause an opposite effect. However, the reactive centres sensitivity to the influence of substituents differs greatly. For balance (I) – pKa(6) = 1.23, and for balance (II) – pKa(1) – pKa(6) = 0.40, i.e. the carboxyl group sensitivity in the ring (B) of 4,5-dymethoxy-N-(2′-carboxyphenyl)anthranilic acids molecules to the influence of substituents is much greater than sensitivity of the same ionogenic group in the anthranilic fragment (A).

The quantitative assessment of the influence of substituents in the nonanthranilic fragment of 4,5-dymethoxy-N-(2′-carboxyphenyl)anthranilic acids (5a-f) molecule has been carried out in the range of the principle of linear free energy (LFE) by the Gamete equation (Fig.).

The pKa indices of all compounds studied were used for correlation because schemes of pKa(I) – f(σ),
The equations I and II obtained have statistically significant parameters:

**Balance I**
\[ \text{pKa}(I) = (6.43 \pm 0.06) - (1.78 \pm 0.06)\sigma \]  
\( n=6 \)  \( s=0.182 \)  \( r=0.997 \)

**Balance II**
\[ \text{pKa}(II) = (6.98 - 0.05) - (0.78 - 0.05)\sigma \]  
\( n=6 \)  \( s=0.154 \)  \( r=0.991 \)

Comparison of the reaction constants \( \rho_I = 1.78 \) and \( \rho_{II} = 0.78 \) shows that sensitivity of the reactive centre I (carboxyl group in the ring B) is 2.3 times higher than sensitivity of the reactive centre II (carboxyl group in the ring A). Probably, it is connected with the different distance between carboxyl groups and substitutes in the ring B. It should be noted that appearance of another reactive centre does not practically influence upon sensitivity (within the limits of experimental error \( \rho_c \) corresponds to \( \rho \) from the other isostructural series of N-phenylantranilic acids) [12, 13, 18-20]. For balance I sensitivity of the reactive centre is sufficiently high because of closeness of substitutes.

It has been found that the substances synthesized have the anti-inflammatory, analgesic, diuretic, bacteriostatic and fungistatic activity. According to the classification by K.K. Sydorov the substances synthesized when introducing intragastrically belong to low-toxic compounds (DL\(_{50} > 3000 \) mg/kg).

**Experimental Part**

Melting points (°C) were measured with the Koeffer’s point apparatus and were not corrected. IR-spectra were recorded by the FT-IR Bruker Tensor 27 spectrometer in KBr. \(^1\)H NMR-spectra were recorded by the Varion Mercury 200 (200 Mhz) spectrometer in DMSO-\(d_6\) using TMS as an internal standard (chemical shifts are reported in ppm).

The derivatives of 4,5-dymethoxy-N-(2’-carboxyphenyl)anthranilic acid were synthesized by a modified Ullmann reaction [1, 3]. The compounds obtained were recrystallized three times from ethanol and dried to 105°C up to the constant weight. The purity of the compounds was checked by thin-layer chromatography.

Reagents. Dioxane used («oscillating») was not purified additionally.

For preparing mixed solutions a fresh boiled bi-distillate liberated from CO\(_2\) was used [21].

The methods of measurements were similar to those described in [21]. 0.05 M aqueous solution of KOH purified from CO\(_2\) served as a titration agent. The concentration of titration solutions was 0.005 mol/l. Potentiometric titration was conducted at 25°C on an EV-74 ionometer using a glass electrode ESP-43-074 and a silver chloride electrode EVL-1 M. The pKa of acetic acid in the binary solvent of dioxane-water solution (60 vol% of dioxane) was determined as a standard (pKa exp. = 7.50; 5.52; 7.49).

The pKa measurements were conducted for each compound independently. The accuracy of the results obtained was estimated by the methods of mathematical statistics (with the reliability level of 0.95) [22].

**Conclusions**

1. The preparative methods for synthesis of 4,5-dymethoxy-N-(2′-carboxyphenyl)anthranilic acids in the solid phase and in the aqueous medium with the use of a phase transfer catalyst – sodium oleate have been developed.

2. Reactivity of 4,5-dymethoxy-N-(2′-carboxyphenyl) anthranilic acids (6 compounds) has been investigated by studying the acid-base balance of these compounds in the binary solvent of dioxane-water.

3. It has been found that 4,5-dymethoxy-N-(2′-carboxyphenyl) anthranilic acids are dibasic subacids,
which strength depends upon the nature and position of substituents.

4. The quantitative assessment of the influence of substitutes on two reactive centres of the acids synthesized has been carried out by the method of correlation analysis according to the Gamete equation.

5. It has been proven that sensitivity of the reactive centres substantially differs and depends on the distance of the substitutes. Herewith, appearance of another reactive centre does not practically influence on sensitivity of the first centre.

6. The correlation equations $pK_{a,1}$ – $f(\sigma)$ obtained for 4,5-dimethoxy-N-(2-carboxyphenyl)anthranilic acids allow to predict acid-base properties of the other compounds of this isostructural series and to use them for QSAR-analysis.

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