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ANALYSIS

QUALITATIVE AND QUANTITATIVE ANALYSIS OF NEW AMINO ACID DERIVATIVES OF ANTICONVULSANT ACTIVITY

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Abstract: Qualitative and quantitative methods for analysis of three new amino–acidic anticonvulsants of low neurotoxicity: pyrazole–3,5–dicarboxylic acid benzylamine (**1**), pyrazine–2–carboxylic acid benzylamine (**2**) and (R, S) N–acetyl–2–pyrrolidone–5–carboxylic acid benzylamide (**3**) have been developed. Qualitative analysis included characteristic reactions, chromatographic (TLC) investigations, IR and UV spectra interpretation and quantitative analysis involved spectrophotometric, chromatographic (HPLC) and acidimetric methods.

Keywords: pyrazole–3,5–dicarboxylic acid benzylamide, pyrazine–2–carboxylic acid benzylamide, (R, S) N–acetyl–2–pyrrolidone–5–carboxylic acid benzylamide, identification, quantitative methods.

Some derivatives of aromatic amides of N–acyl– and N–alkylamino– acids show a strong anticonvulsant activity (1–3). Three aromatic amides, synthesized by one of us, are the representatives for compounds of this group: pyrazole–3,5–dicarboxylic acid benzylamide (**1**), pyrazine–2–carboxylic acid benzylamide (**2**) and (R, S) N–acetyl–2–pyrrolidone–5–carboxylic acid benzylamide (**3**).

Qualitative and quantitative methods for analysis of these three amino–acidic anticonvulsants have been developed on the basis of pharmacopoeial methods of control.

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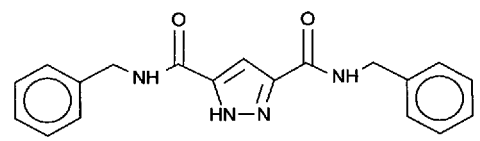

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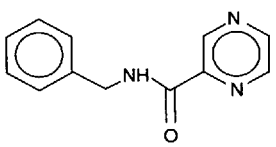

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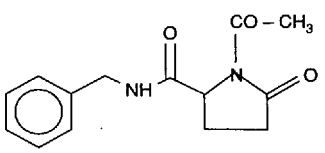

Figure 1. Structures of compounds

EXPERIMENTAL

Materials, apparatus and methods

Compounds (**1, 2** and **3**) – were obtained from the Department of Drug Chemistry (Figure 1). Coloured reactions of compounds with Dragendorff (only **2**), Mayer (only **1**), Millon (only **3**), Nessler reagents as well as with potassium dichromate and nitric acid solutions were developed (Table 1). Melting points of analyzed compounds were determined with Boetius apparatus (Table 2). TLC was carried out on the CD–Alufolien Kieselgel 60 F_{254} plates (Merck) by using the ethanol–water–ammonia (70:30:1 v/v) (A), benzen–ethanol (4:1 v/v) (B), chloroform–methanol (80:20 v/v) solvent systems (Table 3). UV–radiation (254 nm): commercial 1% solutions of Dragendorff reagent was used to visualise the spots. All the three methods are sufficiently sensitive to detect ca. 10 μg of **1, 2** and **3**. UV spectra were taken with an Ultrospec III (Pharmacia LKB) spectrophotometer within the range of 200–800 nm in methanol; IR spectra were recorded by means FTS 135 (BIO–RAD) spectrophotometer (the range of 4000 to 400 cm^{-1} in the solid state) (Table 2).

Quantitative methods of determination of analyzed compounds: spectrophotometry, the HPLC and acidimetry in non–aqueous media were worked out and treated statistically (Table 4). Spectrophotometric and chromatographic methods were

Table 1. Coloured reactions of the analysed compounds

Comp.	Reagents				Solutions of	
	Dragendorff	Mayer	Millon	Nessler	HNO_3	K_2CrO_4
1	–	white	–	white	orange	orange
2	orange	–	–	white	orange	orange
3	–	–	white	white	orange	–

Table 2. Physico-chemical properties of the analysed compounds

Comp.	Formula m.w.	M.p. °C	Solubility according to FP V	IR spectra (cm^{-1}) CO–NH–C	C–H ar.	N–H	C=O	UV–spectra λ max (nm) a 1%, 1 cm
1	$C_{19}H_{18}O_2N_4$ 334,37	284–287	practically insoluble in MeOH, H_2O	3181 3329	1452 1524	1423	1643	210 840
2	$C_{12}H_{11}ON_3$ 213,24	114–115	slightly soluble in MeOH, practically insoluble in H_2O	3375	1522	1456	1671	213,269 860,920
3	$C_{14}H_{16}O_3N_2$ 260,29	122	fairly soluble in MeOH, practically insoluble in H_2O	3299	1455 1498	1554	1648	212 850

validated in terms of precision, linearity, the limit of detection and quantitation.

Spectrophotometric method

Standard solutions in methanol of the following concentrations were prepared: 5,0; 10,0; 15,0; 20,0; 25,0 $\mu g \cdot cm^{-3}$ (for **1** and **3**), 10,0; 20,0; 30,0; 40,0; 50,0 $\mu g \cdot cm^{-3}$ (for **2**). Calibration curve was drawn (λ**1**=210 nm, λ**2**=269 nm, λ**3**=212 nm). 12,5 mg of **1**, **2** and **3** were dissolved in 25,0 cm^3 of methanol: 0,75 cm^3 (**1**, **2**) and 1,50 cm^3 (**3**) of these solutions were taken to the volumetric flask and filled up to 25,0 cm^3. The absorbance of these solutions was measured at 210 nm (**1**), 269 nm (**2**) and 212 nm (**3**).

Results of determinations of compounds showed good precision (coefficient of variation for **1**=0,25%, **2**=0,44% and **3**=0,37%). The limit of detection of the assay for **1**=1,5 μg, **2**=2,5 μg and **3**=0,1 μg, limit of quantitation for **1**=2,5 $\mu g \cdot cm^{-3}$, **2**=10 $\mu g \cdot cm^{-3}$ and **3**=0,2 $\mu g \cdot cm^{-1}$. Linearity for **1** was 2,5–30 $\mu g \cdot cm^{-3}$, **2**=5–70 $\mu g \cdot cm^{-3}$ and **3**=0,2–40 $\mu g \cdot cm^{-3}$.

The HPLC method

The HPLC system Shimadzu (Japan) consisting of pump (LC 10 AT) in conjunction with variable-wavelength UV detector (SPD 10 A) and computer registrator/recorder Chroma (POLLAB, Poland) was used. Rheodyne 7125 valve (Rheodyne Inc. CA. USA) with 50 μl loop was used for injection. The column (150 mm × 4,6 mm) was filled with Ultrasphere ODS (Beckman). The mobile phase was methanol–water (70:30 v/v for **1** and **2**; 65:35 v/v for **3**). The flow rate was 0,7 $cm^3 \cdot min^{-1}$. Retention times obtained in these conditions were: for **1** – 4,79 min (at 210 nm), for **2** – 3,28 min (at 269 nm), for **3** – 4,64 min (at 212 nm).

Calibration curve procedure

Standard solutions were prepared in the mobile phase. Concentrations obtained were as follows: 0,2; 0,4; 0,6; 0,8; 1,0 $\mu g \cdot cm^{-3}$. 50 μl of each sample were then injected into the column.

Table 3. Results of TLC investigations of the analysed compounds

Mobile phase	R_f values		
	1	2	3
A	0,88	0,70	0,82
B	0,70	0,77	0,68
C	0,22	0,40	–

Table 4. The results of quantitative determination of the analysed compounds

Method	1 $\bar{\mu}_{95} = \bar{x} \pm ts$ (%)	2 $\bar{\mu}_{95} = \bar{x} \pm ts$ (%)	3 $\bar{\mu}_{95} = \bar{x} \pm ts$ (%)
Spectrophotometric	100,31 ± 0,27	100,15 ±0,46	100,43 ± 0,39
HPLC	98,99 ± 0,46	100,84 ± 3,51	104,87 ± 2,93
Acidimetric	99,72 ± 0,43	99,74 ± 0,05	100,66 ± 0,39

Determination of **1**, **2** and **3**

The substance (12,5 mg) was dissolved in 25,0 cm^3 of the mobile phase and 0,30 cm^3 of this solution was taken to the volumetric flask and filled up to 25,0 cm^3; 50 µl of each sample was then injected into the column.

Peak areas of compounds were correlated linearly with their concentrations within the tested range (0,2–100 $\mu g \cdot cm^{-3}$ – for **1**, **2** and **3**). The lower limit of detection for **1**, **2** and **3** was 50 ng. Limit of quantitation for **1**, **2** and **3** was 200 $ng \cdot cm^{-3}$. The precision of the chromatographic analysis of **1**, **2** and **3** was determined by the coefficient of variation (for **1**=0,44%, **2**=3,31%, **3**=2,67%).

Acidimetric assay (in non–aqueous medium)

The substance (0,04 g) was dissolved in a mixture of 7 cm^3 glacial acetic acid and 3 cm^3 of acetic anhydride and was titrated with 0,1 $mole \cdot cm^{-3}$ perchloric acid in the presence of crystal violet.

CONCLUSIONS

The set of color reactions is characteristic for analyzed compounds and makes it possible to distinquish them from others. All three quantitative methods of determination are of good accuracy and repeatability and they can be recommended.

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