# THE USE OF 7-(2-BROMOETHOXY)COUMARIN FOR THE QUANTITATIVE FLUOROMETRIC ANALYSIS OF DRUG PREPARATIONS CONTAINING A TERTIARY NITROGEN ATOM

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In drug therapy, especially in the case of long courses of therapy, the content of the drugs in the organism must be monitored. This requires reliable, highly sensitive methods of quantitative determination of drugs in biological material (blood, urine). Moreover, such methods are essential for quality control in their production.

Methods permitting the determination of drugs, accepted by the State Pharmacopoeia of the USSR, 10th edition [1] and described in the scientific literature, as a rule, include various methods of titration [1, 5], photometry [2-4, 6, 9, 10], and chromatography [7, 8, 11, 12].

In our opinion, the method of fluorescent labeling of the preparations to be determined, based on the use of fluorescent reagents, is extremely promising for the identification and quantitative determination of drug agents in biomedia.

In this work we describe the synthesis of one of the derivatives of 7-hydroxycoumarin (I) — 7-(2-bromoethoxy)coumarin (II) — and its use as a fluorescent label for a number of drug preparations containing a tertiary nitrogen atom, for example, dimedrol (III), Cyclosyl (IV), and Glypin (V), which do not possess intrinsic fluorescence.

The synthesis of II was carried out by the reaction of I with dibromoethane in acetone or ethylene glycol in the presence of $K_2CO_3$ at the boiling point of the reaction mixture for 24 h. Under the reaction conditions, 7,7'-ethylenedihydroxybiscoumarin (VI) is also formed.

$$\underset{\text{II}}{R(CH_2)_2Br} \quad \underset{\text{VI}}{R(CH_2)_2} \quad \underset{\text{VII}}{[R(CH_2)_2\overset{+}{N}Me_2(CH_2)_2OCHPh_2]\,Br^-}$$

R = coumarinyl-7-hydroxy

The fluorescent reagent II, in interaction with the bases of the drug preparations III-V, which have a tertiary nitrogen atom, forms the corresponding quaternized compounds of the type of VII (for dimedrol) in dry acetone at 70°C; these compounds are readily soluble in water and have the same fluorescence parameters (except for its intensity) as II.

The coumarin derivatives II and VI, as well as quaternized compounds of dimedrol (VII), Cyclosyl (VIII), and Glypin (IX), were characterized by the data of elementary analysis (Table 1), IR, and PMR spectra (Table 2). In addition, we studied their UV absorption spectra, as well as the excitation and emission spectra. It was established in this case that

TABLE 1. Characteristics of Compounds II, VI-IX

| Compound | Yield, % | mp, °C | Found, % | | | | Gross formula | Calc., % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | H | N | Br | | C | H | N | Br |
| II | 30,5 | 136 | 49,19 | 3,62 | — | 29,65 | $C_{11}H_9O_3Br$ | 49,07 | 3,34 | — | 29,73 |
| VI | 9,0 | 237 | 68,42 | 4,32 | — | — | $C_{20}H_{14}O_6$ | 68,57 | 4,0 | — | — |
| VII | 77,0 | 192 | 64,30 | 5,52 | 2,55 | 15,95 | $C_{28}H_{30}O_4NBr$ | 64,12 | 5,17 | 2,67 | 15,26 |
| VIII | 38,5 | 154 | 60,10 | 5,90 | 2,50 | 14,10 | $C_{28}H_{33}O_6NBr$ | 59,75 | 6,0 | 2,35 | 14,28 |
| IX | 43,0 | 171 | 63,87 | 3,87 | 2,25 | 12,90 | $C_{33}H_{33}O_6NBr$ | 64,24 | 3,38 | 2,38 | 12,78 |

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TABLE 2. Data on the IR and PMR Spectra of Compounds II, VI-IX

| Compound | IR spectra, $\nu_{max}$, $cm^{-1}$ | | | PMR spectra, CDCl, δ, ppm (multiplicity, J, Hz) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OH | C=O | CH=CH | $H_{3,4}$ | $H_{5,6}$ | $H_1$ | O—$CH_2$ | $CH_2$—Br | Ar—CH—Ar or CH—O—CO | $CH_2$—$\overset{+}{N}$ | $\overset{+}{N}$—$CH_3$ | $CH_2$—$CH_2$ | Ar |
| II | 1730 | 1730 | 1615, 1560, 1530, 1500 | 6,26, 7,66 (d, 10) | 7,42, 6,88 (d, 9) | 6,84 (s) | 4,37 (t, 7,5) | 3,66 (t, 7,5) | | | | | |
| VI | | 1735 | 1620, 1565, 1520, 1470 | 6,33, 7,71 (d, 10) | 7,46 6,97 (d, 9) | 6,93 (s) | 4,46 (s) | | | | | | |
| VII | | 1705 | 1625, 1610, 1510, 1495 | 6,33, 8,02 (d, 10) | 7,67, 6,94 (d, 9) | 7,03 (s) | 3,79—4,57 (m) | | 5,61 (s) | 3,51 (s) | 3,22 (s) | | 7,25 7,34 (m) |
| VIII | 3300 | 1720 | 1615, 1570, 1540, 1510 | 6,25. 7,45 (d, 10) | 7,40 (d, 9) 6,85, 6,90 (q, 9,2) | 8,60 (d, 2,5) | 3.50—4,40 (m) | | 5,55 (m) | 3,50 (m) | 3,30 (m) | 2,0—3,2 (m) | 7,25 |
| IX | 3250—3380 | 1715—1740 | 1620, 1605, 1575, 1550, 1510 | 6,29 5,59 6,80 6,92 (q, 9; 2,5) | 7,40 (d 9) | 6,89 (d, 2,5) | 4,5 (m) | | 5,20 (m) | | 3,30 (m) | 2,05—4,70 (m) | 7,25 |

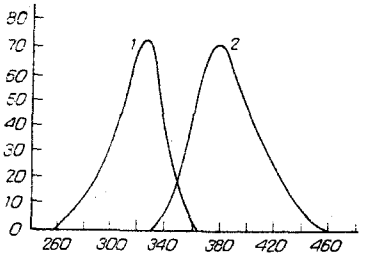


Fig. 1

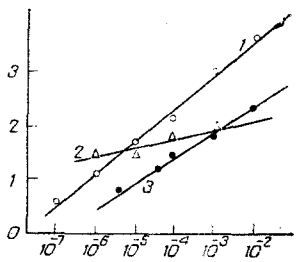


Fig. 2

Fig. 1. Estimation (1) and emission (2) spectra of 7-(2-bromoethoxy)coumarin. Along x-axis: wavelength (in nm); along y-axis: intensity of excitation and emission (in rel. units).

Fig. 2. Dependence of the fluorescence intensity on the concentration of the compounds. 1) VII; 2) VIIII; 3) IX. Along x-axis: concentration (in mg/ml); along y-axis: fluorescence intensity (in rel. units).

compounds II, VI, and VII-IX have absorption maxima in the UV region at 242, 262, and 324 nm, and in the excitation and emission spectra at 325 and 380 nm, respectively (Fig. 1).

Our work shows that when compound II is used as a fluorescent label, the determination of small amounts of the product of the interaction of this compound with the corresponding drug preparation (for example, with VII, VIII, IX) becomes accessible. The detectability of preparations III, IV, and V in this case is down to 0.1 ng. And yet, a combination of the fluorescent method with preparative thin-layer chromatography permits the reliable isolation of individual forms of the quaternized compounds obtained, which carry a fluorescent label (for example, VII, VIII, IX), by which complete specificity of the quantitative determination of one drug preparation or another is achieved. As a result of our work it was established that the most successful solvent system for the chromatographic separation of the reaction products is the system methanol—hydrochloric acid (15:0.2), while for the elution of VII-IX from the chromatogram for subsequent measurements of the fluorescence, distilled water acidified to pH 3.0-4.0 proved the most suitable.

In a study of the dependence of the fluorescence intensity on the concentrations of the investigated compounds VII-IX it was established that a direct dependence is observed in the concentration range $10^{-2}$-$10^{-6}$ mg/ml (Fig. 2).

## EXPERIMENTAL

The UV spectra were recorded on a Perkin-Elmer 402 spectrophotometer (USA) in ethanol, the excitation and emission spectra on a Hitachi MPE-2A spectrofluorometer (Japan) in ethanol and water, the IR spectra on a UR-20 spectrometer (German Democratic Republic) in liquid petrolatum, and the PMR spectra on a Bruker HX-90 MHz spectrometer (Federal Republic of Germany) in $DCCl_3$. The melting points were determined on a Koeffler block, and elementary analysis of the compounds was performed on a Hewlett automatic C,H,N-analyzer (USA). The purity of the synthesized compounds was tested on Silufol plates from Kavalier. For visual observation of the fluorescence of the substances studied on the chromatograms, an illuminator of the BIO-1 UFS-2 type was used. The initial reagents used in the work were purified according to the methods known in the literature.

7-(2-Bromoethoxy)coumarin (II). A 16.2 g (0.1 mole) portion of umbelliferone I was dissolved in 150 ml of acetone, and 18 ml of dibromoethane and 14 g $K_2CO_3$ were added. The reaction mixture was boiled for 24 h. Then the potash was filtered off, the solvent concentrated under vacuum, and the residue chromatographed on a column (5 × 150 cm) with natural $Al_2O_3$ (800 g, degree of activity II). Elution was performed with a mixture of $CHCl_3$ and hexane (or petroleum ether), gradually increasing the polarity of the system. Biscoumarin VI, which was recrystallized from DMFA and 8 g of the bromide II, which was crystallized from benzene, were obtained. II is a white substance, readily soluble in organic solvents, insoluble in water; it possesses bright blue fluorescence (excitation at λ 325 nm, emission at λ 380 nm).

Compound VII. To a solution of 0.4 g (0.0025 mole) II in 50 ml of dry acetone we added 0.5 g (0.002 mole) of dimedrol base, heated the reaction mixture at 70°C for 6 h, and then

TABLE 3. Determination of Cyclosyl in Water Using the Bromide II

| No. | Taken, mg/ml | Found,* mg/ml |
|---|---|---|
| 1 | $1 \cdot 10^{-3}$ | $9 \cdot 10^{-4}$ |
| 2 | $1 \cdot 10^{-4}$ | $9 \cdot 10^{-5}$ |
| 3 | $1 \cdot 10^{-5}$ | $8 \cdot 10^{-6}$ |
| 4 | $1 \cdot 10^{-6}$ | $8 \cdot 10^{-7}$ |

*Here and in Table 4, the data are averages of five measurements, relative error 6-10%.

TABLE 4. Determination of Dimedrol in Rabbit Blood Serum (*in vitro*) and Glypine in Rat Blood Serum (*in vitro*) with the Use of the Bromide II

| Preparation | Dose, mg/ml | Found* in serum, mg/ml |
|---|---|---|
| Dimedrol | 30 | $3 \cdot 10^{-4}$ |
| Glypin | 10 | $8 \cdot 10^{-5}$ |

left it at ~20°C for 24 h. Crystals thereupon precipitated; they were filtered off and washed twice with acetone. Yield 0.6 g of compound VII.

Compound VIII. To a solution of 0.2 g (0.0007 mole) II in 15 ml of dry acetone we added 0.2 g of cyclosyl base. Then the process was conducted analogously to VII. Yield 0.15 g of VIII.

Compound IX. To a solution of 0.72 g (0.003 mole) II in 25 ml of dry acetone we added 0.7 g (0.002 mole) glypine. Then the process was conducted analogously to VII. The yield was 0.72 g IX.

## QUALITATIVE AND QUANTITATIVE DETERMINATION OF DRUGS USING REAGENT II

For the identification and subsequent quantitative determination of the preparations studied in water or blood serum, 5-10 ml of liquid was placed in a separatory funnel, the pH of which was adjusted to 10.0-11.0 with 1 N NaOH, mixed thoroughly, and $CHCl_3$ immediately extracted three times with 30 ml portions for 3-4 min. The chromophore extracts were combined, transferred to a 150 ml round-bottomed flask, and the solvent distilled off to dryness under vacuum. Then 1 ml of a standard solution (concentration 0.1 mg/ml) of II in dry acetone was added to the flask with the extract. The volume was adjusted to 5 ml with dry acetone, then the reaction mixture was thermostatically controlled at 56-58°C for 1 h, after which acetone was evaporated under vacuum to dryness, and the residue dissolved in 0.5 ml ethanol. A 0.2 ml portion of the solution obtained was applied with a micropipette on the starting line of a Silufol plate. The dried plate was placed in a preliminarily (5-10 min) saturated chamber with the solvent system methanol—hydrochloric acid (15:0.2). When the solvent front is at a distance of 0.5 cm from the upper edge, the plate is extracted from the chamber, the length of the front is noted, and it is dried in an exhaust hood for 10-15 min. The spots are detected by examination of the chromatogram in UV light. In this case the investigated compound appears in the form of a bright blue spot. $R_f$ values: for VII 0.43, for VIII 0.38, and for IX 0.50, which permits their identification among numerous natural compounds, contained in the investigated objects. After this, the corresponding spots are cut out, and the substances are washed off the plate with 10 ml of distilled water acidified to pH 3.0-4.0, through a paper filter.

The fluorescence of the solution is measured on a spectrofluorometer from Hitachi MPF-2A at the wavelength 380 nm, exciting the fluorescence by light with wavelength 325 nm. The

quantitative content of the investigated compounds is determined according to calibration curves (see Fig. 2) or by the method of comparing the fluorescence intensities of standard solutions of quaternized compounds with those of the test samples. Certain data obtained in the determination of the parameters studied are cited in Tables 3 and 4.

Thus, we are suggesting a method of qualitative and quantitative determination of certain drugs containing a tertiary nitrogen atom, with the aid of the fluorescent reagent II.

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