Chemistry of Natural Compounds, Vol. 43, No. 2, 2007

QUANTITATIVE DETERMINATION BY PMR SPECTROSCOPY OF LAGOCHILIN IN THE SUBSTANCE AND TABLETS OF THE MEDICINAL PREPARATION INEBRIN

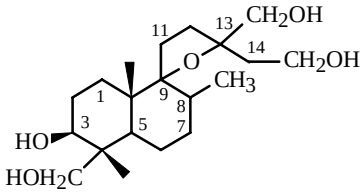
Kh. M. Bobokulov,[1] M. G. Levkovich,[1] A. Kh. Islamov,[2] U. N. Zainutdinov,[3] and N. D. Abdullaev[1]

UDC 547+543.062:543.429.25

A modified method of additions in PMR spectroscopy was described for quantitative determination of the main active principle in preparations. The effectiveness and reliability of the method was approved for quantitative analysis of the active principle lagochilin in the substance (2.0%) and tablets (0.85%) of the commercial medicinal preparation inebrin.

Key words: *Lagochilus*, lagochilin, medicinal preparation inebrin, quantitative determination, PMR spectroscopy, method of additions.

Lagochilin (**1**) and its derivatives are isolated from plants of the genus *Lagochilus* (Lamiaceae) and are promising compounds for preparing pharmacologically active preparations, in particular, hemostatic agents [1-3]. One of these is the medicinal preparation inebrin, the dry extract of the plant as tablets, which is an effective hemostatic with a sedative and antiallergic effect that decreases the circulation time and improves the blood supply [4].



1

The goal of the research was to develop an effective method for determination and quality control of the lagochilin content directly in the substance and tablets of inebrin. The need to develop this method using PMR spectroscopy was due to difficulties with performing this analysis by high-performance liquid chromatography and other classical methods for such measurements of lagochilin.

Lagochilin is extracted by pyridine very effectively (practically 100%) and with few associated compounds from inebrin substance or its powdered tablets. This enables direct qualitative and quantitative determination of lagochilin in the extract of inebrin by comparison of PMR spectra of solutions in deuteropyridine of pure lagochilin and of inebrin extract. The signal of the C-4 methyl protons (0.96 ppm) turned out to be convenient for quantitative determinations of **1**. The standard resonance for measuring its intensity was the signal of residual α-protons of deuterated pyridine (α-Py), which was also the solvent for recording the PMR spectra.

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 700170, Tashkent, fax (99871) 120 64 75, e-mail: khayrulla@rambler.ru; 2) A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, 700143, Tashkent, ul. Kh. Abdullaeva, 83; 3) Mirzo Ulugbek National University of Uzbekistan, 700174, Tashkent, Vuzgorodok, fax (99871) 144 77 28. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 124-126, March-April, 2007. Original article submitted October 31, 2006.

0009-3130/07/4302-0149

TABLE 1. Relative Amplitudes and Integrated Intensities of Measured Signals*

Sample	**1**	$I(CH_3)/I_{\alpha\text{-Py}}$	$S(CH_3)/S_{\alpha\text{-Py}}$	$I_{HMDS}/I_{\alpha\text{-Py}}$
1	0.99	2.57	1.72	1.27
3	0.89	2.27	1.53	1.19
4	0.77	2.02	1.36	1.27
5	0.65	1.71	1.22	1.23
6	0.56	1.40	1.01	1.21
7	0.47	1.31	1.00	1.13
8	0.41	1.16	0.83	1.14
9	0.34	1.02	0.76	1.17
10	0.29	0.91	0.69	1.17
11	0.23	0.76	0.62	1.11
2	0.0	0.33	0.34	0.97

*The intensity of the pyridine α-proton signal was taken as 1.

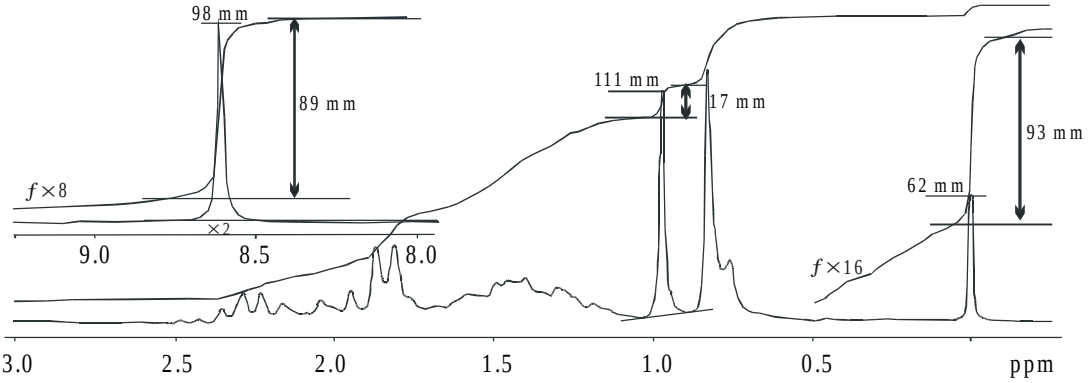


Fig. 1. Region of interest of lagochilin PMR spectrum during titration of extract of inebrin substance (sample No. 3).

However, it should be noted that the width of signals in the PMR spectrum of standard lagochilin and lagochilin in inebrin extract can differ because of the presence in the latter of associated impurities. The different width of the signals, in turn, can lead to noticeable errors in the quantitative measurements if the signal intensities in their spectra are compared directly. In our instance, the concentration of **1** in inebrin by such direct measurements of the intensities of this methyl was 2.4%; according to integrated intensities, 0.46%. The latter result can be explained by distortion of the measured integrated intensities on the background of impurity signals.

In order to increase the accuracy and reliability of the measurements, we used a modification of the method of additions, which we proposed earlier for use in PMR spectroscopy [5]. In this version of the method of additions, the usual titration of the measured preparation with a control is repalced by the reverse process. The solution of the control preparation (standard **1**) is diluted in several steps by a solution of the analyzed compound up to a zero concentration of the starting control preparation, i.e., a successive transition is made from the standard solution to the measured preparation. Next, a least-squares analysis of the change of amplitude or integrated intensity of the measured signals (in our instance the lagochilin C-4 methyl protons) as a function of relative concentrations of the control and measured solutions is made. The quantitative content of **1** in the analyzed substance is calculated from these data.

A special solvent of deuteropyridine and hexamethyldisiloxane (HMDS) with approximately the same intensities for the proton signals of α-Py and HMDS was prepared for the measurements and additional monitoring of the accuracy of them. Then the ratio of intensities of these signals was used to monitor the reliability of the recorded PMR spectra. For accurate measurements, the intensity of the HMDS signal should remain constant during titration of lagochilin by inebrin and provide additional evidence that the measured signal intensities are correct. If a linear dependence of this ratio on the relative lagochilin:inebrin concentration is observed, it would be necessary to consider also signal broadening because of some components in the inebrin extract.

The concentrations of the working solutions were 0.99% for lagochilin (sample 1) and 4.99% for inebrin (sample 2). Amplitudes and integrated intensities of the analyzed signal were measured. First PMR spectra were recorded for both working solutions (samples 1 and 2), then for nine dilutions of lagochilin with inebrin extract. All dilutions were made by weight on an analytical balance. Only the region of interest of the spectra was recorded (Fig. 1). Amplitudes (I) and integrated intensities (S) of the α-Py signal, the analyzed signal, and HMDS were measured (Table 1).

Assuming a linear dependence of the methyl signal intensity (Y) on the concentration ratio of samples **1** and **2** (X), the coefficients of the equation for this dependence and their mean-square deviations were calculated, where $A = 0.250 \pm 0.031$, $B = 2.262 \pm 0.052$ (for measured signal amplitude), and $Y = A + B \cdot X$.

The variable X is defined as the fraction of the starting lagochilin concentration in the working solution, i.e., in the starting sample (n = 1), X = 1. The value of X decreases in the titrated samples (n = 3-11) whereas X = 0 in sample n = 2.

The lagochilin concentration in the starting sample 1 is proportional to the sum of coefficients A + B (X = 1). In analyzed sample 2, it is proportional only to A (X = 0). The lagochilin concentration in analyzed sample 2 is established from this ratio by proportion using

$$C_X = 0.99 \times [0.250/(0.250 + 2.262)] = 0.099.$$

Because this amount of lagochilin in sample 2 corresponds to 4.99 wt. % of the sample weight, the concentration of lagochilin itself is

$$C = (0.099\%/4.99\%) \times 99.5\% = 1.97 \pm 0.24\%.$$

Measurement of integrated intensities gave the elevated erroneous result $C = 3.44 \pm 0.24\%$. This was due to complications with the correct measurement of the signal intensity, which overlaps signals of impurities (subtracting the baseline from measured amplitudes is easier than from integrated intensities). Because the expected result according to certificate data was 2.0%, it is obvious that use of measured amplitudes in the proposed version of the method of additions is perferred over the commonly accepted integrated intensities.

The last column in Table 1, as already noted, is designed to estimate the uncertainty in the proposed method itself. Ideally the ratio $I_{HMDS}/I_{\alpha\text{-Py}}$ should be constant. In our instance, this ratio is 1.17 ± 0.049, i.e., the linear dependence of this ratio on the quantity X can be neglected (the deviation 0.049 is small enough relative to the value itself). The random uncertainty of the method can be estimated as $(0.049/1.17) \cdot 100\% = 4.2\%$ of the calculated value.

The efficiency of lagochilin extraction from inebrin was checked by pouring the remaining extract solution with the precipitate onto filter paper and drying. The dry residual was extracted again with deuterated pyridine for 2 h. The PMR spectrum of the second extract under conditions analogous to the first determination showed no lagochilin in the second extract. Therefore, lagochilin, which is very soluble in pyridine, was totally extracted from inebrin substance during the first extraction.

The lagochilin content in inebrin tablets is halved. The content of lagochilin in powdered inebrin tablets that was measured by the described method gave a lagochilin concentration $C = 0.85 \pm 0.08\%$.

Thus, a slightly modified version of the method of additions is proposed for the most accurate quantitative measurements by PMR spectroscopy. A solution of the active compound is titrated by the analyte. The quantitative measurement is made by recording the same signal in a series of spectra. This decreases the level of several systematic errors. The method allows the use of standard methods of statistical treatment of the experimental data and gives results together with confidence intervals and the reliability of the measurements.

The proposed method can increase the accuracy of actual measurements by 2-4 times.

EXPERIMENTAL

All experiments were performed on a Tesla BS-567A spectrometer at working frequency 100 MHz for ^1H. Samples were dissolved in deuteropyridine (99.7% deuterated). The internal standard was HMDS, the chemical shift of which was taken as 0.06 ppm. Spectra were recorded at room temperature. Samples were not degassed. Therefore, even narrow signals had a width of >1 Hz for experiments with a resolution of about 0.5 Hz. The signal of residual pyridine α-protons (0.03% remaining

after deuteration) at 8.58 ppm was used as the reference signal. The pulse sequence (power and duration of exciting pulse, relaxation time, number of scans, etc.) was strictly repeated in all experiments. Because the relaxation time for similar samples was fractions of a second, the delay between pulses of 3 s and more enabled relaxational distortions of signal intensities to be neglected. Also, the control signal of HMDS could be analyzed by ignoring the dependence of signal widths on relative concentrations of the working solution. The range of amplitudes of this narrow signal and, therefore, its width, was only 4.2%. For broader actual PMR lines, such additional broadening can be completely neglected because it will be even smaller. The number of scans for measuring relative intensities of PMR signals was not important. However, a sufficient number (more than 16-20) is recommended for stability. Additional line broadening using a digital filter is recommended in order to avoid the effect of the Fourier digital parameters of the NMR spectra. In this instance, an exponential smoothing filter with line broadening by 1.0 Hz was used to increase the accuracy and stability of the amplitude measurements. According to recommendations in the literature [6] (the measured line should be defined by at least five points), this was also entirely sufficient for correct integrated measurements.

REFERENCES

1. Z. I. Mavlyankulova, U. N. Zainutdinov, and Kh. A. Aslanov, *Khim. Prir. Soedin.*, 46 (1977).
2. Z. I. Mavlyankulova, U. N. Zainutdinov, F. G. Kamaev, and Kh. A. Aslanov, *Khim. Prir. Soedin.*, 82 (1978).
3. M. P. Nurmatova, U. N. Zainutdinov, F. G. Kamaev, and Kh. A. Aslanov, *Khim. Prir. Soedin.*, 788 (1979).
4. U. N. Zainutdinov, T. K. Yunusov, S. A. Mavlyanov, and M. P. Pulatova, in: *Scientific Materials, International Workshop on Biotechnology Commercialization and Security*, Tashkent (2003), 83.
5. Kh. M. Bobokulov, M. G. Levkovich, and N. D. Abdullaev, *Uzb. Khim. Zh.*, **4**, 41 (2005).
6. E. Deroum, *Modern NMR Methods for Chemical Research*, Mir, Moscow (1992).