

8. R. C. Cambie, P. K. Grant, C. Huntrakul, and R. J. Weston, *Aust. J. Chem.*, **22**, 1691 (1969).
9. H. R. Shenk, H. Gutman, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **35**, 817 (1952).

DEVELOPMENT OF A METHOD FOR DETERMINING BORNYL ACETATE IN THE PRODUCTS OF THE PROCESSING OF *Abies sibirica*

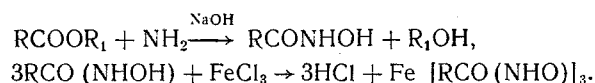
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A number of investigations has been published recently on the development of a technology for the production of extracts from coniferous raw material [1]. The main component of the essential oil of *Abies sibirica* (Siberian fir), responsible for the smell of the oil, is bornyl acetate [2].

Several methods of determining the amount of esters are used: saponification [3], gas chromatography [2], and crystallization out from the essential oil (for bornyl acetate) [2]. The standard method of saponification based on the titration of the acids, is inapplicable to a CO₂ extract of the fir, since the latter has a pronounced color and titration of the acids does not show a distinct change in the color of the indicator. The gas chromatographic method is unsuitable for working with small amounts of CO₂ extracts, since these extracts contain a large amount of nonvolatile substances, and working with small samples does not enable the amount of nonvolatile fraction to be determined. Crystallization out from the essential oil cannot be used in the case of CO₂ extracts because the presence of waxes, sterols, and resin acids in the CO₂ extracts imparts a viscous consistency to it which interferes with the formation of crystals and is associated with large losses.

We have used the reaction of esters with an alkaline solution of hydroxylamine [4]. This reaction forms hydroxamic acids. The product of the reaction of these acids with salts of trivalent iron gives the solution a pink-yellow color (pH 2.0 ± 0.2). The reaction takes place in accordance with the equations



EXPERIMENTAL

By using a scheme for the planning of experiments by the method of steepest ascent, we found the optimum conditions for performing the reaction [5]. As the optimization parameter we used the optical density of the colored product Y formed. As the independent variables we chose the following:

- X₁—the amount of hydroxylamine hydrochloride, ml;
- X₂—the amount of NaOH, ml;
- X₃—the time for the first stage of the process, min;
- X₄—the amount of HCl, ml;
- X₅—the amount of FeCl₃, ml;
- X₆—the time for the second stage of the process, min.

We used a 20% solution of hydroxylamine hydrochloride, a 0.5 N solution of NaOH, a 5 N solution of HCl, and a 6% solution of FeCl₃ prepared in 0.1 N hydrochloric acid. All the solutions were used in the freshly prepared state. The planning matrix for the experiment and the results are shown in Table 1.

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TABLE 1. Planning Matrix of the Experiment and the Results

Independent variables.	x_1	x_2	x_3	x_4	x_5	x_6	\bar{y}_D
Upper level	1,0	3	15	1,5	3	10	
Lower level	0,5	2	10	0,5	2	5	
Range of variation	0,25	0,5	2,5	0,5	0,5	2,5	
Expt. 1	+	+	+	+	+	+	0,13
2	+	+	+	+	+	+	0,13
3	+	+	+	+	+	+	2,12
4	+	+	+	+	+	+	0,21
5	+	+	+	+	+	+	0,20
6	+	+	+	+	+	+	0,14
7	+	+	+	+	+	+	0,36
8	+	+	+	+	+	+	0,17
Regression coefficient bi	-0,281	+0,269	-0,216	-0,223	-0,255	+0,207	
Step (f)	-0,07	+0,15	+0,50	-0,11	-0,13	+0,5	
Expt. 1	0,43	3,15	15,5	0,39	1,87	10,5	0,13
2	0,36	3,30	16,0	0,28	1,74	11,0	1,47
3	0,29	3,45	16,5	0,17	1,61	11,5	1,15
4	0,22	3,60	17,0	0,06	1,48	12,0	0
5	0,15	3,75	17,5	0	1,35	12,5	0

Note. The steepest ascent was performed after dilution (threefold) in comparison with the first series of experiments.

First we performed a series of experiments with planning in accordance with the scheme of a fractional 2^{6-3} experiment. After the calculation of the regression coefficients we determined the step in the direction of steepest ascent and performed a series of experiments in the direction of steepest ascent.

The steepest ascent procedure gave the following optimum values of the variables : $X_1 = 0.36$; $X_2 = 3.3$; $X_3 = 16.0$; $X_4 = 0.28$; $X_5 = 1.7$; $X_6 = 11.0$.

For the determination of the esters, about 0.5 g of the extract was dissolved in 25 ml of ethanol. The ethanolic solution was passed through a column of alumina (activity grade II) to free it from the pigments which it contained. The irreversible adsorption of the esters on the column was 11%. To compensate these losses, a correction coefficient averaging 1.11 must be introduced into the calculation.

To 5 ml of the solution obtained were added 0.36 ml of the hydroxylamine hydrochloride solution and 3.3 ml of the NaOH solution. The solutions were stirred and allowed to stand for 16 min, and then 0.28 ml of the HCl solution and 1.7 ml of the FeCl_3 solution were added. The mixture was stirred, and after 11 min its optical density was determined on an FÉK-56 photocolormeter with the fifth filter. A blank experiment was performed in parallel. A calibration curve was plotted for bornyl acetate at concentrations of 0.2-2.2 mg.

We recorded the spectra of the products of the interaction of ferric chloride with the esters of the CO_2 extract, the essential oil, and a reference sample (bornyl acetate) on an SF-4 spectrophotometer in ethanol in the visible region (Fig. 1). The spectra of the bornyl acetate, the CO_2 extract, and the essential oil each have two maxima in the wavelength ranges 400-450 nm (1) and 500-550 nm (2). The coincidence of the maxima of the bornyl acetate, the CO_2 extract, and the essential oil permitted this region to be used for determining the optical density of these substances.

Comparative results of the determination of the amount of esters in a CO_2 extract of the Siberian fir by the proposed method and the standard method, calculated as bornyl acetate, are given below (%):

Expt. No.	Amount of Esters According to the Proposed Method	Amount of Esters According to the Standard Method
1	15.53	12.07
2	15.16	13.63
3	16.36	13.00
4	16.21	—
5	16.54	—
Mean	15.96	12.9

The relative error [5] of the proposed method is 4.5%, and that of the standard method 9.7%.

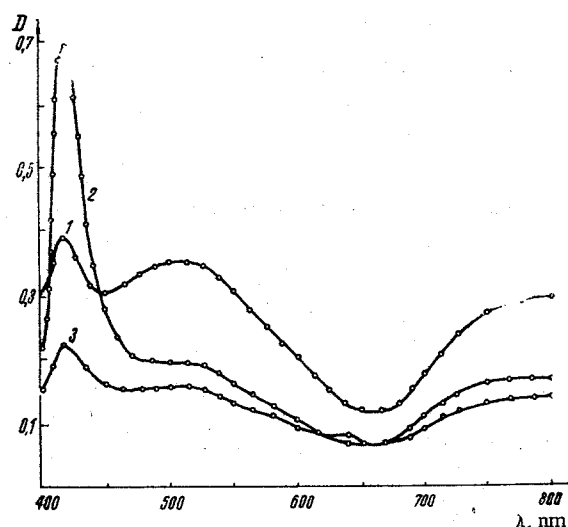


Fig. 1. Spectral characteristic of the products of the reaction of ferric chloride with the esters of the CO₂ extract, the essential oil, and bornyl acetate: 1) bornyl acetate; 2) essential oil; 3) CO₂ extract.

In view of the great diversity of the esters in plant materials and their inadequate availability, we performed a comparative determination of the esters calculated as bornyl acetate and as ethyl acetate, and calculated a conversion factor of 4.88.

Comparative results of the determination of the esters calculated as bornyl acetate and as ethyl acetate are given below (%):

Expt. No.	Amount of Esters Calculated as Bornyl Acetate	Amount of Esters Calculated as Ethyl Acetate
1	15.53	$3.20 \times 4.88 = 15.61$
2	15.16	$3.08 \times 4.88 = 15.03$
3	16.36	$3.32 \times 4.88 = 16.20$
4	16.21	$3.34 \times 4.88 = 16.29$
5	15.54	$3.41 \times 4.88 = 16.74$
Mean	15.96	$3.27 \times 4.88 = 15.96$

The use of ethyl acetate as a reference sample permits the proposed method to be used under the conditions of a factory laboratory.

SUMMARY

The conditions for the reaction of esters—bornyl acetate—with an alkaline solution of hydroxylamine have been studied by the method of steepest ascent. A method has been developed for determining esters in a CO₂ extract of Abies sibirica.

LITERATURE CITED

1. A. V. Pekhov, N. F. Lyuban'kova, S. S. Morozova, and A. N. Katyuzhanskaya, *Izv. Sev. Kav. Nauchnogo Tsentra Vysshei Shkoly*, No. 4, 20 (1974).
2. M. Goryaev and M. Pliva, *Methods of Investigating Essential Oils* [in Russian], Alma-Ata (1962), p. 634.
3. GOST 14618.7-69: *Essential Oils, Odorous Substances, and Intermediates of Their Synthesis. Methods of Testing* [in Russian].
4. E. A. Peregud and E. V. Chernet, *The Chemical Analysis of the Air of Industrial Enterprises* [in Russian], Moscow-Leningrad (1963).

5. V. V. Nalimov and N. A. Chernova, Statistical Methods of Planning Extremal Experiments [in Russian], Moscow (1965).

PRODUCTION OF A PYRIDINE DERIVATIVE OF GROSSHEMIN

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Information has appeared in recent years on new sesquiterpene lactones isolated from plants. The poor solubility of the sesquiterpene lactones in water complicates their biological testing and, consequently, their practical use. In view of this, attempts have been made to convert the sesquiterpene lactones into soluble compounds. Thus, for example, for some compounds containing an α -methylene- γ -lactone ring derivatives with primary and secondary amines have been obtained which, in their turn, give water-soluble salts [1, 2]. The reaction of sesquiterpene lactones with tertiary amines has not been established.

We report the preparation of a pyridine derivative of grosshemin, which is formed by the reaction of pyridine and hydrochloric acid with grosshemin (I) in the presence of acetic anhydride.

The pyridine derivative of grosshemin (II), $C_{20}H_{24}O_4NCl \cdot H_2O$, mp 160–165°C, $[\alpha]_D^{20} + 71.23^\circ$ (c 1.33; water) is readily soluble in water, soluble in ethanol, and sparingly soluble in other organic solvents.

The pyridine derivative is formed only in the presence of acetic anhydride, which is a catalyst in this reaction. Grosshemin acetate (III) does not form a pyridine derivative, and the pyridine derivative of grosshemin is acetylated only under more severe conditions.

The IR spectrum of (II) has absorption bands at (cm^{-1}) 3450 (OH) and 3300 (H_2O), 1770 (γ -lactone), 1745 (cyclopentanone), and 1640 and 1620 (double bonds in conjugation).

The NMR spectrum of (II) (Fig. 1) has the following signals (ppm): doublet at 1.11–3 H_{15} ; triplet at 3.96– H_4 ; multiplet at 4.28– H_6 ; two singlets at 4.54 and 4.85–2 H_{14} ; quartet at 5.64 ($J_{13-13'} = 13.8$ Hz; $J_{11,13} = 9.0$ Hz)– H_{13} ; quartet at 5.89 ($J_{13,13'} = 13.8$ Hz; $J_{11,13'} = 5.0$ Hz)– $H_{13'}$; two triplets at 8.02 (2 H) and 8.40 (1 H); and a doublet at 9.90 (2 H)–pyridine protons.

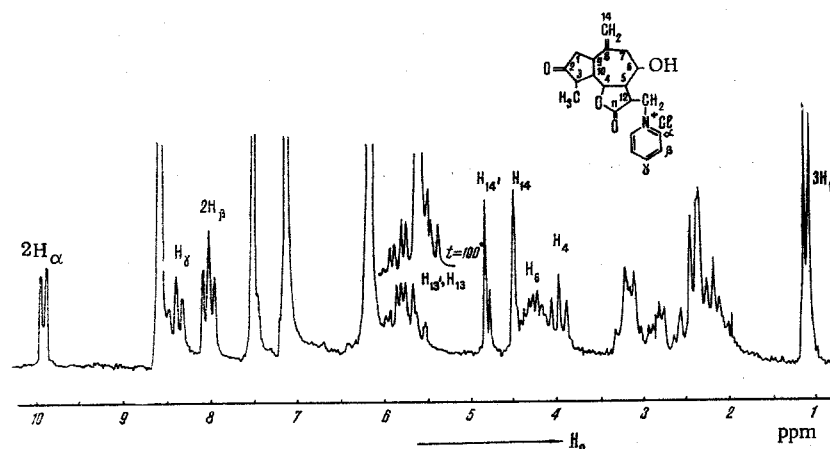


Fig. 1. NMR spectrum of the pyridine derivative of grosshemin in d-pyridine.

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