A STUDY OF THE REACTION OF PENTACYCLIC TRITERPENOIDS WITH CONCENTRATED SULFURIC ACID

V. F. Semenchenko, É. T. Oganesyan, V. D. Ponomarev, and V. I. Frolova

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We have previously reported that, on being dissolved in concentrated sulfuric acid, pentacyclic triterpenoids form substances having, without isolation from the reaction medium, a characteristic maximum at 310 nm [1]. The conditions of performing this reaction given by various authors are different, and there is no single opinion on this [2-8]. Considering the analytical possibilities of this reaction, we set ourselves the task of studying the optimum conditions for its performance. In order to determine the optimum conditions of the reaction of pentacyclic triterpenoids with concentrated sulfuric acid, we used one of the methods of the mathematical planning of experiments – the method of steepest ascent with respect to the response surface [9]. The initial conditions and results of the first series of experiments are given in Table 1. The experiments were carried out on samples of oleanolic and ursolic acids, which belong to different groups of triterpenoids – the α- and β-amyrin groups. The results of the reactions are given in the form of log ε. The experimental results permitted the coefficients of the regression equation to be calculated from the formulas [9]

$$b_0 = \frac{\sum y}{n}; \quad b_i = \frac{\sum x_i \cdot y}{n}; \quad b_{ij} = \frac{\sum x_i \cdot x_j \cdot y}{n},$$

where x is the coded value of the variables x_1 and x_2; b_{ij} is the coefficient for the effect of interaction; n is the number of experiments; b_0 is the free member of the equation; y is the result of the reaction; and b_i is the coefficient for the variable.

The following values of the regression coefficients were obtained:
for oleanolic acid

$$b_0 = 3.817; \quad b_1 = +0.016; \quad b_2 = +0.087; \quad b_{1,2} = +0.0060.$$
$$y = 3.817 + 0.016\,x_1 + 0.087\,x_2 + 0.060\,x_1x_2;$$

for ursolic acid

$$b_0 = 3.813; \quad b_1 = 0.021; \quad b_2 = +0.054; \quad b_{1,2} = +0.130.$$
$$y = 3.813 + 0.021\,x_1 + 0.054\,x_2 + 0.0130\,x_1x_2.$$

The significance of the regression coefficients was evaluated from the formulas

$$Sb_i = \sqrt{\frac{S^2y}{n}}; \quad \Delta b_i = t \cdot Sb_i.$$

For oleanolic acid, $S_y = 0.0033$; $Sb_i = 0.0016$; $\Delta b_i = 0.0051$ (here all the regression coefficients are significant); for ursolic acid $S_y = 0.0050$; $Sb_i = 0.0024$; $\Delta b_i = 0.0076$ (all the regression coefficients are significant); S_y was determined by the usual methods [9].

The values of the regression coefficients obtained permitted the following conclusions to be drawn: log ε rises with a rise in the temperature (b_1 in this case is significant and has a + sign) and with an increase in the reaction time (b_2 is significant in both cases and has a + sign).

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TABLE 1. Levels of the Factors and the Planning Matrix

Characteristic	Temp., °C (x_1)	Time, min (x_2)	Result of the reaction (log ε): ursolic acid (y_2)	Result of the reaction (log ε): oleanolic acid (y_1)
Zero level	50	20	—	—
Range of variation	20	12	—	—
Upper level +1	70	32	—	—
Lower level −1	30	8	—	—
Experiments				
1	+1	+1	4,020	3,980
2	+1	−1	3,650	3,687
3	−1	+1	3,716	3,828
4	−1	−1	3,868	3,774

TABLE 2. Steepest Ascent with Respect to the Response Surface

Characteristic	Ursolic acid			Oleanolic acid		
	x_1	x_2	y_2	x_1	x_2	y_1
Zero level	50°	20min	—	50°	20min	—
Range of variation	20°	12min	—	20°	12min	—
Coefficient	+0,021	0,054	—	0,016	0,087	—
Product	0,42	0,648	—	0,32	1,044	—
Step	6°	10min	—	3,2°	10min	—
Experiments						
1	50°	20min	3,959	50°	20min	3,900
2	55°	30min		55°	30min	
3	60°	40min	3,965	60°	40min	3,920
4	65°	50min		65°	50min	
5	70°	60min	4,050	70°	60min	4,159
6	75°	70min	4,052	75°	70min	4,162

Consequently, to achieve the optimum conditions for the reaction it is necessary to increase its temperature and time. For this purpose a steepest ascent with respect to the response surface was performed (Table 2). Of the calculated steps, experiments 1, 3, 5, and 6 were reproduced. In experiments 5 and 6 the optimum region was achieved, since a further increase in the temperature and the time caused an insignificant improvement in the result of the reaction. The measurement of the optical density of the solution in the 12 hours after the beginning of the experiment showed that the optical density remained unchanged.

Thus, the optimum conditions for performing the reaction of triterpenoids with concentrated sulfuric acid may be regarded as heating the solutions at 70°C for 60 min. Below we give an example of the calculation of the coefficients for oleanolic acid.

$$b_0 = \frac{3.980 + 3.687 + 3.828 + 3.774}{4} = 3.817;$$

$$b_1 = \frac{(+1)\,3.980 + (+1)\,3.867 + (-1)\,3.828 + (-1)\,3.774}{4} = 0.016 \text{ and so on.}$$

EXPERIMENTAL

A measuring flask (20 ml) with a ground-in stopper was charged with a sample (0.001 g) of oleanolic acid. A sample (0.001 g) of ursolic acid was placed in a flask of the same capacity. To each flask was added 10 ml of concentrated sulfuric acid (d 1.835), and the contents of the flask were shaken until the substance had dissolved completely. After this, 0.5-ml samples of the colored sulfuric acid solutions were taken from each flask, their volume was made up to 10 ml with sulfuric acid, and they were thermostatted at various temperatures and for various times according to the planned matrices of the experiments (see Tables 1 and 2). The optical densities of the solutions were determined in an SF-4A spectrophotometer at a wavelength of 310 nm (layer thickness in the cells 10 mm) relative to pure sulfuric acid.

SUMMARY

The conditions for the reaction of triterpenoids with concentrated sulfuric acid have been studied by the method of mathematical planning, and the optimum conditions for performing this reaction have been found.

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