## METHODS OF ANALYSIS AND QUALITY CONTROL

## STEROIDS

XLVI. A METHOD OF CLEAVAGE OF SOLASODINE TO 5,16-PREGNADIENE-3 $\beta$ -OL-20-ONE ACETATE. ANALYSIS OF INTERMEDIATE PRODUCTS

L. I. Klimova, G. G. Malanina,

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L. M. Morozovskaya, N. N. Suvorov, E. M. Peresleni, and Yu. N. Sheinker

We reported earlier [1] that in the industrial synthesis of dehydropregnenolene acetate (I) from solasodine, at the step of oxidation of the O,N-diacetate of  $\psi$ -solasodine (II), the yield of the oxidation product (III) is 70-75%, whereas at the remaining steps the yields reach 90-95%. In view of the fact that the production of I is a multi-ton industry, it seems important to study the possibility of optimization of the oxidation process. This necessitates the development of methods of analysis of the initial and final products of this reaction. In this article we describe methods of analysis of II and  $16\beta$ -( $\delta$ -acetylamino- $\gamma$ -methylvalerianoxy)-5-pregnen- $3\beta$ -ol-20-one (III).

It was established that II adds 1 mole of iodine, probably at the double bond in the 20,22-position. This peculiarity of II has permitted the use of a method of iodometric titration for its quantitative determination. The method was developed on an analytically pure sample of II. In the development of the analysis, optimum ratios of the sample weight of II and amount of 0.1 N solution of iodine were selected, and the influence of the temperature system of titration was verified. It was shown that variation of the temperature in the range 15-40° has no effect on the results of the titration. The accuracy of the determination was es-

Sample weight (in g)	Composition of mixture	Calculated, %		Found, %		Error (in
		II	111	II	111	% abs.)
0,2010	0,1403 II 0,0607 III	69,8		69,15		0,65
0,2139	0,0896 I 0,0715 II 0,0528 III	33,4		32,6		0,8
0,0796	0,0094 I 0,0399 II 0,0301 III		37,9		34	3,9
0,0799	0,0102 I 0,0199 II 0,0497 III		62,2		58	4,2
0,6992	0,0989 I 0,3013 II 0,2990 III	43,09	42,7	43,04	38	0,05 4,7

TABLE 1. Determination of the % Content of II and III in Artificial Mixtures

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Fig. 1. Graph of the dependence of the % content of III on the optical density of the solution.

timated according to the method of [2]. The confidence interval is  $100.28 \pm 0.48$  at the 99% probability level, and  $100.29 \pm 0.32$  at the 95% probability level.



IV and I may be present as impurities in addition to III in the reaction mixture after the oxidation of II. Compound I is formed as a result of elimination of the side chain of III. It has been established experimental-

ly that compounds I, III, and IV do not add iodine. This permits the quantitative determination of II to be conducted in the mixture of products formed in its oxidation. The determination of II was conducted in ar-tificially compiled mixtures: double (II + I) and triple (II + III + I). The percent content of II, determined by titration, is in sufficiently good agreement with that set.

For an analysis of III in the reaction mixture after oxidation, we used the method of R spectroscopy. In the IR spectrum of III, an absorption band is observed at  $1180 \text{ cm}^{-1}$ , which was absent in the spectra of I, II, and IV. The IR spectra were taken in the region of  $1080 \text{ cm}^{-1}$  for 13 artificially compiled mixtures of III + II in a solution of chloroform at 5-10% intervals. A graph of the dependence of the optical density of the solution on its content of III was constructed on the basis of the data obtained. This dependence is linear at contents of III up to 90%, which indicates its obedience to the Beer-Lambert law. The calibration straight line is cited in Fig. 1. To verify the reproducibility of the results, we determined III in artificially compiled mixtures are cited in Table 1.

<u>Methods of Determinations</u>. The analytical sample II has the following characteristics: mp 134-138°, R<sub>f</sub> 0.29 (silufol, chloroform – acetone 9:1). Found %: C 74.77; H 9.52; N 3.07.  $C_{31}H_{47}$  NO<sub>4</sub>. Calculated %: C 74.81; H 9.52; N 2.81. The following results were obtained in titration of the sample: 100.7, 100.5, 99.7, 100.5, 100.6, 100.4%, and in titration of a sample with an addition of the acetic acid, 100.3, 100.6, 100.1%. For the analytical sample III, see [3].

Determination of the Percent Content of II in a Dry-Weighed Sample. About 0.2 g (exact-weighed sample) of the substance was placed in a 250 ml flask with a ground stopper, dissolved in 30 ml of alcohol, 25 ml of a 0.1 N solution of iodine was added, the solution shaken and left in darkness for 20 min. Then 30 ml of chloroform and 100 ml of water were added to the solution and the excess iodine titrated with a 0.1 N solution of sodium thiosulfate (starch indicator). A parallel control experiment was conducted; 1 ml of the 0.1 N iodine solution corresponded to 0.02488 g II.

Determination of the Content of II in Acetic Acid Solution (Step of Isomerization). A 20-ml portion of the acetic acid solution of II used for the step of oxidation was collected with a pipette, a solution diluted to 100 ml (in a volumetric flask) with glacial acetic acid, and 5 ml of this solution collected with a pipette for determination. The further analytical procedure was as described above.

Preparation of a Sample of the Reaction Mixture after Oxidation for Analysis. A 5-6-ml portion of the acetic acid solution after oxidation with sodium bichromate was collected and poured out into 10 ml of a 5% solution of sodium sulfate; the mixture was extracted twice with 20 ml portions of benzene. The benzene extract was washed with water, with a diluted solution of sodium bicarbonate, again with water, and dried with anhydrous magnesium sulfate. The magnesium sulfate was filtered off, washed with benzene, and the benzene solution evaporated to dryness under vacuum. The residue in the flask was dried to constant weight, after which it was used for the determination of II and III. <u>Compound II</u> was determined as described for a dry-weighed sample. For the determination of III a 4% solution of the mixture in purified chloroform was prepared, and the spectrum in the region of 1100-1250 cm<sup>-1</sup> was recorded on a UR-10 instrument at a layer thickness of 0.28 mm with pure chloroform as a transparent standard. The optical density was determined at 1180 cm<sup>-1</sup> (maximum of the absorption band of III) and the percent content of III found according to the calibration straight line.

## LITERATURE CITED

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