ISATIN HYDRAZONE – A DETECTING REAGENT FOR 3-KETOSTEROIDS

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Drugs of the class of 3-ketosteroids possess a broad action spectrum [1]. These substances are prescribed in small doses which, of course, causes certain difficulties in determining their authenticity both as such and in complex medicinal forms.

The reagents used for detecting 3-ketosteroids [2] have a number of serious disadvantages connected with the complexity of the procedure and, moreover, the sensitivity of the individual procedures is between 20 and 50 μg of steroid in a sample [2, 3].

Our aim was to increase the sensitivity of detection and to simplify the procedure for determining drugs of the 3-ketosteroid class by the TLC method on the basis of their reaction with isatin hydrazone.

For analysis we used drugs in pure form (Table 1) corresponding to the demands of the USSR State Pharmacopoeia tenth edition [4], or other standardizing technical documentation, in the form of 0.001 M dioxane solutions. The isatin hydrazone was used in the form of a 1% dioxane solution acidified with 5% HCl (0.4 ml of HCl to 100 ml of solution). Solutions with lower concentrations (see Table 1), which were still detected by the reagent on a chromatogram, were obtained by diluting the initial solutions of 3-ketosteroids with dioxane. The experimentally established limit of detection of the compounds studied ($\mu g \cdot 10^{-4}$) was between 0.36 (prednisolone) and 2.07 (deoxycorticosterone trimethylacetate). In all cases the 3-ketosteroids were revealed in the form of orange spots on a yellow background of a Silufol UV-254 plate. The hR_f values are given in Table 1. The procedure developed has been used for the qualitative identification of a number of 3-ketosteroids and has been tested in the analysis of medicinal forms.

On Silufol UV-254 plates at a distance of 1 cm from an edge 1-μl portions of dioxane solutions of 3-ketosteroids of definite concentration obtained by diluting the initial 0.001 M solutions were deposited with the aid of a regulatable manual microdosing device. The distance between the spots on the chromatograms was 1.5 cm. Chromatography was carried out at 18-20°C. The distance migrated by the solvent was 10 cm. The chromatograms were dried

TABLE 1. hR_f Values and Limits of Detection ($\mu g \cdot 10^{-4}$) of Some 3-Ketosteroids

3-Ketosteroid	System						Limit of detection
	1	2	3	4	5	6	
Ethisterone	54	28	58	43	18	33	0,39
Methyltestosterone	60	24	54	44	15	30	1,51
Hydroxyprogesterone caproate	66	24	63	48	9	29	0,54
Deoxycorticosterone trimethyl acetate	56	59	57	48	12	28	2,07
Prednisolone	61	19	58	51	12	21	0,36
Hydrocortisone acetate	55	23	58	47	10	22	0,41
Progesterone	59	24	54	45	12	25	1,57
Hydrocortisone hemisuccinate	64	19	55	47	12	17	1,61
Deoxycorticosterone acetate	72	19	65	58	16	16	1,86
Testosterone propionate	67	22	63	51	16	18	0,42
17-α-Hydroxyprogesterone	64	22	60	46	11	20	0,41

Chromatographic systems: 1) benzene–dioxane (2:1); 2) benzene–ethanol (9:1); 3) benzene–isopropanol (4:1); 4) chloroform–acetone (3:1); 5) chloroform–ethanol (98:2); 6) benzene–acetone (4:1).

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and treated with a 1% dioxane solution of isatin hydrazone, after which they were heated at 100°C for 5-10 min.

LITERATURE CITED

1. M. D. Mashkovskii, Drugs [in Russian], Moscow, Part 1 (1985), pp. 564, 598.
2. J. G. Kirchner, Thin Layer Chromatography, Wiley-Interscience, New York, 2nd edn. (1978) [Russian translation, Mir, Moscow, Vol. 1 (1981), p. 221].
3. I. M. Hais and K. Macek, Paper Chromatography, Academic Press, New York, 3rd edn. (1963).
4. State Pharmacopoeia of the USSR [in Russian], Moscow (1968).

EPISTEROL AND FUNGISTEROL FROM MUTANT STRAINS OF THE YEAST *Saccharomyces cerevisiae*

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Mutant strains of the yeast Saccharomyces cerevisiae having blocks in the stages of the biosynthesis of ergosterol accumulate its intermediates. The use of nystatin-resistant mutants permits the sequence of stages in sterol synthesis to be established and strains to be obtained that accumulate sterols suitable for transformation into biologically active substances [1]. We have obtained a strain of the yeast Saccharomyces cerevisiae with mutations simultaneously in the two genes NYS3 and NYS4. Mutations in the NYS4 gene prevent the introduction of a double bond into the C22(23) position, and those in the NYS3 gene block dehydrogenation in the C5(6) position.

Gas-chromatographic analysis showed that the cells of the strains with mutations simultaneously in the two genes NYS3 and NYS4 accumulate two sterols the retention times of which exceed that of ergosterol. We succeeded in establishing the structures of these sterols by the method of mass spectrometry, which is widely used for these purposes [2]. The sterols were identified on the basis of the characteristics of fragmentary ions with values of m/z $\gg$ 200. It must be mentioned that many yeast sterols give identical fragmentary ions, and therefore for their identification the ratio of the intensities of the peaks must be taken into account. The sterols of the NYS3 and NYS4 strains have molecular ions with m/z 400 and 398. Their structures apparently differ by the number of double bonds. The peaks of the ions with m/z 385 and 383 correspond to the splitting out of a methyl group. The $[M - CH_3]^+$ ion is formed by the detachment of a methyl group both from C10 and from C13. The presence of a basic peak with m/z 271 in the spectrum indicates that double bonds are present both in the side chain and in the nucleus. The $[M - R]^+$ and $[M - R - 2H]^+$ ions are characteristic for Δ^5- and Δ^7-sterols with one double bond in the side chain [3]. The ratio of the intensities of the peaks with m/z 271 and 314 determines the position of the double bond in the nucleus. The m/z 271 peak is more intense than the m/z 314 peak if the sterol has a Δ^7-double bond [3]. High-intensity peaks of the $[M - R - 2H]^+$ and $[M - R - H_2O]^+$ ions are characteristic for Δ^5- and Δ^7-sterols [4]. However, the spectrum lacks the peaks characteristic for Δ^5- and $\Delta^{5,7}$-sterols that are connected with the breakdown of the tetracyclic nucleus [5]. The intensities of the peaks also permit the position of a double bond in the side chain to be determined. Intense peaks with m/z 257 and 255, and also a triplet of low-intensity peaks with m/z 299, 300, and 301 indicate that the double bond is present in the C24 position [3]. The fact that the intensity of the m/z 255 peak is greater than that of the m/z 213 peak indicates the presence of a Δ^{24}-bond in the side chain not suppressing fragmentation in ring D but leading to a predominance of dissociation with the formation of a m/z 255 ion [6]. Consequently, it may be concluded that the ergostane derivative with a molecular mass m/z 398 and two double bonds in the C7 and C24 positions is ergosta-7,24(28)-dien-3β-ol (episterol).

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