SYNTHESIS OF DERIVATIVES OF THE ANDROSTANE SERIES. XXI. SYNTHESIS AND PROPERTIES OF SOME N-ACYLATED ANDROSTANO[3,2-c]PYRAZOLES

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A method has been developed for synthesizing acylsteroidopyrazoles in which the acyl residues are radicals of natural amino acids. The compounds synthesized possess a high anabolic action and a low toxicity.

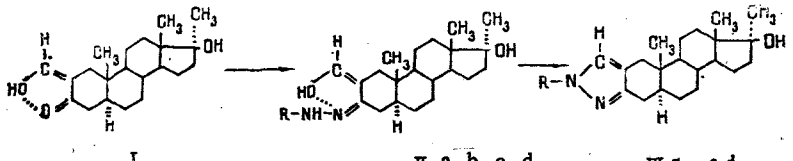
A special place among biologically active substances in the series of heterocyclic derivatives of the androstane series is occupied by the androstano[3,2-c]pyrazoles [1], among which 17β-hydroxy-17α-methylandrostano[3,2-c]pyrazoles have been among the most active protein-anabolic drugs for about 20 years.

The study [2] of N-derivatives of androstano[3,2-c]pyrazoles has shown that only N' acyl derivatives possess an anabolic index no lower than those for the initial substances used. The authors concerned obtained N-acylsteroidopyrazoles by the action anhydrides on the corresponding steroidopyrazoles in pyridine.

It has been established previously [3, 4] that N'acylandrostanopyrazoles can be obtained by the interaction of amino acid hydrazides with a hydroxymethyleneketosteroid. Their formation takes place in two stages: First an hydroxymethylenehydrazone is obtained and this then undergoes dehydration and ring closure to give a N'-acylpyrazole ring. The N'-(p-aminobenzoyl) 17β-hydroxy-17α-methyl-5α-androstano[3,2-c]pyrazole obtained by this method possesses an anabolic index 1.5 times greater than that of the initial androstane derivative.

The present paper is devoted to the synthesis and study of N'-acylsteroidopyrazoles in which the acyl residues are the radicals of four amino acids: phenylalanine, tryptophan, tyrosine, and glutamic acid.

These compounds were obtained by the reaction of 2-hydroxymethylene-17α-methyldihydrotestosterone with the hydrazides of the amino acids by the following scheme [12]:



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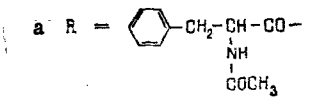

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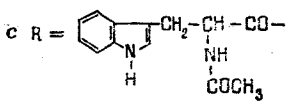

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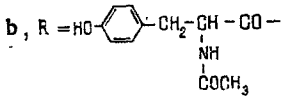

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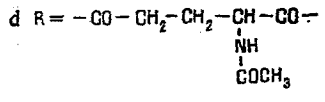

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TABLE 1. Physicochemical Characteristics of 3-N'-Acylhydrazones of 2-Hydroxymethylene-17β-hydroxy-17α-methylandrostanes

Name of the 3-hydrazone	Yield	mp	UV spectrum	IR spectrum, cm^{-1}
IIa, 3-N'-Acetylphenylalanylhydrazone of 2-hydroxymethylene-17β-hydroxy-17α-methylandrostane	99,3	125—127°	232—234 nm	OH – 3420; NH of sec. amide – 3310; C=O of sec. amide – 1670; C=C, C=N – 1510, NH of sec. noncyclic amide – 1510, $COCH_3$ – 1350
IIb, 3-N'-Acetyltyrosylhydrazone of 2-hydroxymethylene-17β-hydroxy-17α-methylandrostane	99,5	168—173°	227 nm	OH in arom. ring – 3500, OH in ring D – 3400, NH of sec. amide – 3320, C=O of sec. amide – 1660, C=C, C=N – 1525; $COCH_3$ – 1355.

As can be seen from Table 1, the IR and UV spectra of compounds (IIa) and (IIb) had the absorption maxima characteristic for hydrazones.

The IR spectra of substances obtained after the condensation of N-acetyltryptophan hydrazide and of N-acetylglutamic acid dihydrazide with the 2-hydroxymethylene-3-ketosteroids showed that there were also bands of the stretching vibrations of the pyrazole ring – 1640-1600, 1550-1470, and 1450 cm^{-1}. This indicates that in this case the reaction does not stop at the stage of the formation of the hydrazone but, in parallel with it the second, dehydration, reaction takes place with the formation of steroidopyrazoles. In view of the fact that the hydrazones (IIc) and (IId) were not isolated in the pure state but were produced in the form of mixtures with compounds (IIIc) and (IIId), the mixtures were subjected to thermal dehydration without separation, which led to the conversion of the whole of the mixtures into the corresponding pyrazoles (IIIc), and (IIId).

The displacement of absorption maximum in the UV part of the spectrum for substance (IIIb) in the long-wave direction (278-280 nm) is explained by the presence of the indole ring[8].

In order to study the possibility of obtaining N'-acylsteroido[3,2-c]pyrazoles, the 3-hydrazones isolated were subjected to thermal dehydration in vacuum [9].

In this way we synthesized N'-acyl-5α-androstano[3,2-c]pyrazoles (Table 2) with amino acid residues and showed that the mechanism of their formation agrees with that for steroidopyrazoles unsubstituted at the nitrogen [9].

The androgenic and anabolic activities were studied by the method adopted by Eisenberg and Gordon [10]. The anabolic index was calculated by means of Hershberger's formula [11]. The results of the investigation are given in Table 3.

It can be seen from Table 3 that N'-acetyltyrosyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole and N'-acetylglutaminylbis(17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole) correspond in their anabolic activities to androstanazole. N'-Acetylphenylalanyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole and N'-acetyltryptophyl-17β-hydroxy-17α-methylandrostano [3,2-c]pyrazole possess a higher anabolic activity. After their administration, the weight of a mouse increased more distinctly than after androstanazole. The androgenic properties of the above-mentioned commpounds are lower than those of androstanazole and only N'-acetylphenylalanyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole is distinguished by a more pronounced anodrogenic action, as a result of which the anabolic index of this compound is somewhat lower than that of androstanazole, while for the other substances tested it is higher. The highest anabolic index is characteristic of N'-acetyltryptophanyl-17β-hydroxy-17α-methylandrostano [3, 2-c]pyrazole, which possesses a more pronounced anabolic effect and a lower androgenic effect than androstanazole.

The toxicity of the substances synthesized was studied in acute experiments on male mice weighing 18-22 g. The compounds described and androstanazole were injected intramuscularly in ethanol—oil solution. Control animals were given only the solvent in the same volume. Observations were made on the animals for 30 days. The results of these investigations are given in Table 4.

TABLE 2. Physicochemical Constants of the N'-Acylsteroido-[3,2-c]pyrazoles

Name of the steroidopyrazole	Yield	mp	UV spectrum	IR spectrum, cm^{-1}
IIIa. N'-acetylphenylalanyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	95.9	140—144°	230—234 nm	OH — 3420; NH of a sec. amide — 3300; CO — 1730, 1670, C=C, C=N — 1610, pyraz. ring 1590, 1550, 1510, 1450, $COCH_3$ — 1340
IIIb. N'-Acetyltyrosyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole	98.1	174—178°	226—227 nm	OH — 3430; NH of sec. amide — 3300; CO — 1730, 1670; C=C, C=N — 1530; pyraz. ring — 1600, 1480, 1453; $COCH_3$ — 1355
IIIc. N'-Acetyltryptophanyl-17β hydroxy-17α-methyl-androstano[3,2-c]pyrazole	99.3	172—175°	270—280 nm	OH — 3420; NH of sec. amide — 3320; CO of tert. amide 1670; C=C, C=N — 1530; pyraz. ring — 1600, 1470, 1450; indole ring — 1600, 1470, 1350, 1020, 920; $COCH_3$ — 1350
IIId. N'-Acetylglutaminyl-bis-17β-hydroxy-17α-methylandrostano[3,2-c]-pyrazole	94.2	194—200°	234—236 nm	OH — 3430, NH of sec. amide — 3300; CO of tert. amide 1660; C=C, C=N — 1640; pyraz. ring — 1640, 1550, 1505, 1450; $COCH_3$ — 1340.

TABLE 3. Androgenic and Anabolic Activities of the N'-Acyl Derivatives of Steroidopyrazoles

Substance	Number of animals	Weight of the organs, mg $(\bar{x} + S\bar{x})$			Anabolic index, AI
		prostate	seminal vesciles	MLD	
IIIa. N'-Acetylphenylalanyl-17β-hydroxy-17α-methylandrostano[3,2-c]-pyrazole	13	33±1,06 P_1<0,001 P_2<0,02	73±8,4 <0,001 <0,001	58±3 3 <0,001 <0,001	0,66
IIIb. N'-Acetyltyrosyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	13	25±1,3 P_1<0,001 P_2<0,02	35±2,0 <0,001 <0,05	45±1,9 <0,001 >0,1	1,17
IIIc. N'-Acetyltryptophyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	12	25±0,9 P_1<0,001 P_2<0,011	34±3,3 <0,001 <0,05	50±0,8 <0,001 <0,001	1,44
IIId. N'-Acetylglutaminyl-di(17β-hydroxy-17α-methylandrostano[3,2-c]-pyrazole)	13	27±2,3 P_1<0,001 P_2<0,1	29±1,7 <0,001 >0,001	40±3,8 <0,001 >0,1	1,28
Androstanazole [stanozolol] 17β-hydroxy-17α-methyl androstano[3,2-c]pyrazole	23	29±1,0 P_1<0,001	42±3,0 <0,001	42±2,0 <0,001	0,80
Control.	30	17±1,3	11±0,37	17±2,4	

For androstanazole the LD_{50} calculated by the method of Litchfield and Wilcoxon amounts to 3700 mg/kg.

As can be seen from Table 4, the toxicities of all the proposed group of compounds are extremely low.

EXPERIMENTAL

The results of elementary analyses of the compounds described corresponded to the calculated figures.

N'-Acetylphenylalanyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole (IIIa). A solution of 2 g of 2-hydroxymethylene-17α-methyldihydrotestosterone and 1.6 g of N'-acetylphenylalanine hydrazide in 20 ml of methanol was boiled under reflux for 5 h. The cooled solution

TABLE 4. Toxicities of the N'-Acyl Derivatives of Steroidopyrazoles in Acute Experiments on Mice

Substance	LD_{50}, mg/kg
IIIa. N'-Acetylphenylalanyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	8100
IIIc. N'-Acetyltryptophyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	2500
IIIb. N'-Acetyltyrosyl-17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole	3000
IIId. N'-Acetylglutaminyldi(17β-hydroxy-17α-methyl-androstano[3,2-c]pyrazole)	1650
Androstanazole	3700

was diluted with 300 ml of saturated NaCl solution and the mixture was allowed to stand until the following day. Then the precipitate was heated with ether (30 ml) for 15 min, filtered off, and washed several times with ether. This gave 3.2 g of 3-N'-acetylphenylalanylhydrazono-2-hydroxymethylene-17β-hydroxy-17α-methylandrostane (IIa) with mp 125-127°C.

The 3-hydrazone obtained (2.89 g) was heated at an oil bath temperature of 140-150°C and a pressure of 5-7 mm Hg for 6 h. After dehydration, 2.63 g (95.9% of theoretical) of N'-acetylphenylalanyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazone (IIIa) with mp 137-139°C was isolated.

N'-Acetyltryptophyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole (IIIc). A mixture of 1.06 g of 2-hydroxymethylene-17α-methyldihydrotestosterone, 1 g of N-acetyltryptophan hydrazide, and 20 ml of methanol was treated in the same way as for the preceding compound. This gave 1.62 g of a mixture of the hydrazone and the pyrazole. After heating in vacuum under conditions similar to those for compound (IIIa), 1.6 g of the mixture obtained yielded 1.54 g of N'-acetyltryptophanyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole (IIIc) with mp 174-178°C.

N'-Acetyltyrosyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole (IIIb). A mixture of 1.16 g of 2-hydroxymethylene-17α-methyldihydrotestosterone, 1 g of N-acetyltyrosine hydrazide, and 20 ml of methanol was heated for 5 h. After a similar working up procedure, 2.00 g of 3-hydrazone with mp 168-173°C was isolated.

The 3-hydrazone (IIc) (1.67 g), after dehydration in vacuum at 140-150°C yielded 1.58 g of N'-acetyltyrosyl-17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole (IIIb) (98.1%), with mp 174-178°C.

N'-Acetylglutamylbis (17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole) (IIId). Under similar conditions, 1.3 g of 2-hydroxymethylene-17α-methyldihydrotestosterone, 1 g of N-acetylglutamic acid dihydrazide, and 20 ml of methanol yielded 1.840 g of a mixture of the hydrazone and the pyrazole with mp 170-172°C. After dehydration, 1.59 g of this mixture yielded 1.460 g (94.2% of theoretical) of N'-acetylglutamylbis(17β-hydroxy-17α-methylandrostano[3,2-c]pyrazole) (IIId) mp 194-200°C.

SUMMARY

1. It has been established that N-acylandrostano[3,2-c]pyrazoles in which the acyl residues are radicals of natural amino acids are formed by the interaction of 2-hydroxymethyleneketosteroids with acetylamino acid hydrazides via the corresponding hydrazones followed by dehydration with closure of the pyrazole ring. It has been shown that for some of the hydrazones synthesized the dehydration reaction takes place in parallel with their formation, and after heating with the amino acid hydrazide a mixture of hydrazone and pyrazole is isolated.

2. An investigation of the androgenic and anabolic effects of the N-acylandrostanopyrazoles synthesized has shown that these compounds possess a high anabolic effect and a low toxicity.

LITERATURE CITED

1. R. O. Clinton, A. J. Manson, and F. W. Sonner, J. Am. Chem. Soc., 83, 1478 (1961).
2. R. O. Clinton and E. Greenbush, U.S. Patents Nos. 3,398,140 (1968) and 3,704,295 (1972)).
3. L. N. Volovel'skii and I. I. Kuz'menko, Zh. Obshch. Khim., 40, 507 (1970).
4. L. N. Volovel'skii and I. I. Kuz'menko, Zh. Obsbch. Khim,, 43, 406 (1973).
5. L. Bellamy, Infrared Spectra of Complex Molecules, 2nd edn., Methuen, London/Wiley, New York (1958).
6. K. Nakanishi, Infrared Absorption Spectroscopy. Practical, Holden-Day San Francisco (1962).
7. A. R. Katritzky, Physical Methods in the Chemistry of Heterocyclic Compounds, Academic Press, New York (1963).
8. K. W. Bentley, Elucidation of Structures by Physical and Chemical Methods Interscience, New York (1963). p. 121.
9. L. N. Volovel'skii, I. I. Kuz'menko, and N. V. Novikova, Zh. Obshch. Khim., 39 (1969).
10. E. Eisenberg and G. S. Gordon, J. Pharm. Exp. Ther., 99, 38 (1950).
11. Z. E. Hershberger, E. G. Shupley, and R. Meyler, Proc. Soc. Exp. Biol. Med., 83, No. 1, 175 (1953).
12. L. N. Volovel'skii, I. I. Kuz'menko, S. N. Ushenko, and N. M. Khvorova, USSR Inventor's Certificate No. 517,589; Byull. Izobret., No. 22, 74 (1976).

THE STUDY OF COMPLEX MIXTURES OF NATURAL SUBSTANCES BY THE DEFOCUSING AND DADI METHODS.

II. PHYTOSTEROLS ACCOMPANYING β-SITOSTEROL

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Low- and high-resolution mass spectra and defocusing and DADI spectra of samples of β-sitosterol isolated from various plant material have been studied. The spectral information obtained has shown the presence in samples of β-sitosterol studied of minor accompanying components which have been identified as stigmasterol, campesterol, and cholesterol.

Phytosterols form a set of C_{27}-C_{29} sterols participating in the structure of cell membranes and in that part of plant metabolism which leads to the formation of phytosterols of higher plants the most widespread is β-sitosterol, which is frequently accompanied by a number of other minor sterols the isolation and identification of which by the usual method present great difficulties.

Continuing our investigation [1] of complex mixtures of natural substances by the defocusing and DADI methods [2, 3], we have studied a number of samples isolated from various plant materials and identified by all their constants [4] as β-sitosterol. Their mass spectra were identical. As an example we give the mass spectrum of a sample isolated from *Lagochilus inebrians* B. (family Labiatae) (Fig. 1). The establishment by the defocusing and DADI methods of a complete genetic link between the ions has shown that the mass spectrum of the sample contains, in addition to the peak of the molecular ion (M^+) with m/e 414 of β-sitosterol (I), the M^+ peaks of another three substances with m/e 412 (II), 400 (III), and 386 (IV) having independent fragmentation pathways.

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