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SYNTHESIS OF MODIFIED ISOFLAVONES AND THEIR ANABOLIC PROPERTIES

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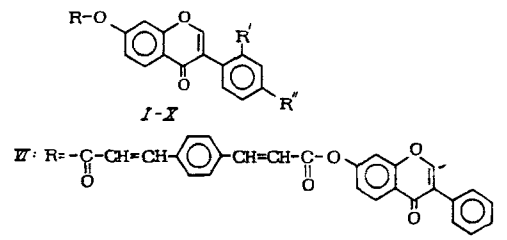
The search for new highly effective drugs in the series of modified isoflavonoids is very promising in view of their availability. Large amounts of isoflavonoids are contained in leguminous plants, which provide excellent fodder for agricultural animals. They have a positive effect on the increase in weight of the animals and the increase in their productivity. Addition of certain isoflavones in an amount of 0.0001 to 0.1% to the animal fodder results in an increase in their weight, and in horses in the strengthening of the musculature. Aminomethylisoflavones cause a 5.74-8.76% increase in weight of chicks in the course of 35 days. These effects are brought about by a nonestrogenic action, and the liver function is thus not disturbed. The increase in weight in domestic animals (pigs, poultry, cattle, sheep) is probably due to the fact that isoflavones activate the synthesis of proteins and lipid metabolism [3].

Being essentially ingredients of fodder of plant origin, many isoflavones are virtually devoid of toxicity. Such a fortuitous combination of low toxicity and high biological activity makes them extraordinarily promising for use in medicinal practice as drugs with anabolic action.

In the present work, we synthesized new derivatives of isoflavones I-X for the purpose of studying their growth-promoting activity.



I-X

V: R = -C(=O)-CH=CH-C6H4-CH=CH-C(=O)-O-

R=H(I—III), $CH(CH_3)_2$ (IV, VII, IX), $COCH{=}CHC_6H_5$ (V), $CH(CH_3)COOCH_3$ (VIII, X); R′=H (I, III—VI, IX, X), F (II, VII, VIII); R″=H (I, II, IV—VIII), F (III, IX, X).

There are several synthetic approaches to the preparation of isoflavones. In the view of the authors of [5], the simplest and most effective is the method consisting in the action of acetic-formic anhydride on 2-hydroxydesoxybenzoins in the presence of triethylamine. The nonavailability of a commercial acetic-formic anhydride prompted us to develop methods circumventing this drawback. The method consisting in the reaction of 2-hydroxydesoxybenzoins with the Vilsmeier reagent in the presence of boron trifluoride etherate [1] makes it possible to obtain quantitative yields in the synthesis of isoflavones; moreover, dimethylform-amide and phosphorus chlorides necessary for the preparation of the Vilsmeier reagent are readily available.

Samples of isoflavones I, III and IV obtained in the present work were identical with the samples obtained by other methods [2, 5, 8]. This is confirmed by the data of quantitative elemental analysis, PMR spectroscopy, and also the melting points of individual and mixed samples. The course of the reaction and the purity of the compounds obtained were monitored by

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TABLE 1. Properties of Modified Isoflavones I-X

Compound	Yield, %	mp, °C	Empirical formula	PMR spectral data, δ, scale, ppm (TMS – internal standard)							Solvent
				isoflavone protons						modifying fragment protons	
				2-H	3-C_6H_4R'	5-H	6-H	8-H	7-OH	7-RO	
I	95	213—215	$C_{15}H_{10}O_3$	8,53	7,57 (R′=H)	8,01	6,96	6,89	10,92		DMSO-d_6
II	98	223—224	$C_{15}H_9FO_3$	8,51	7,28 (R′=F)	8,21	7,05	6,9	10,92		DMSO-d_6
III	98	250,5	$C_{15}H_9FO_3$	8,47	7,49 7,05 (R″=F)	8,0	6,85	6,8	10,89		DMSO-d_6
IV	96	115	$C_{18}H_{16}O_3$	7,86	7,6 (R=H)	8,16	6,93	6,84	—	4,66 (1H, heptet CH) 1,39 (6H, d, J=6Hz, $(CH_3)_2$)	$CDCl_3$
V	79	210—212	$C_{24}H_{16}O_4$	8,55	7,55 (R′=H)	8,58	7,88	7,67	—	8,18 (1H, d, J=15,5Hz α-CH) 6,82 (1H, d, J=15,5Hz, β-CH) 7,55 (5H, s, β-C_6H_5)	CF_3COOD
VI	88	287—289	$C_{42}H_{26}O_8$	8,57	7,56 (R′=H)	8,57	8,60	7,59	—	8,57 (2H, s, 2′-H, 5′-H) 7,56 (5H, s, 3′-C_6H_5) 8,60 (1H, d, J=8 Hz, 2 Hz, 6′-H) 7,59 (1H, d, J=2 Hz, 8′-H) 8,20 (2H, d, J=15,5Hz α-CH, α′-CH) 6,93 (2H, d, J=15,5Hz β-CH, β′-CH)	CF_3COOD
VII	93	124—125	$C_{18}H_{15}FO_3$	7,95	7,28 (R′=F)	8,18	6,94	6,88	—	4,66 (1H, heptet CH) 1,39 (6H, d J=6 Hz $(CH_3)_2$)	$CDCl_3$
VIII	95	158	$C_{19}H_{15}FO_5$	8,24	7,3	8,09	7,03	7,03	—	5,18 (1H, q, J=6,5 Hz, CH) 3,76 (3H, s, OCH_3) 1,66 (3H, d, J=6,5 Hz CH_3)	$(CD_3)_2CO$
IX	92	143—144	$C_{18}H_{15}FO_3$	7,84	7,49 7,05 (R″=F)	8,14	6,87	6,80	—	4,66 (1H, heptet CH) 1,39 (6H, q J=6Hz, $(CH_3)_2$)	$CDCl_3$
X	96	126	$C_{19}H_{15}FO_5$	8,29	7,7 7,1 (R″=F)	8,13	7,09	6,9	—	5,17 (1H, q, J=6,5 Hz, CH) 3,76 (3H, s, OCH_3) 1,65 (3H, d, J=6,5 Hz, CH_3)	$(CD_3)_2CO$

TABLE 2. Values of Acute Toxicity of Compounds I – X (n = 6)

Compound	LD_{50}, mg/kg	Confidence interval
I	2430	1850—3010
II	2520	1920—3120
III	2500	2010—2990
IV	3185	2850—3520
V	3850	2500—5200
VI	5140	3000—7280
VII	2975	1750—4200
VIII	2710	2010—3410
IX	3100	1800—4400
X	7200	2080—3320
DIT	1110	880—1340

TABLE 3. Anabolic Effect of Modified Isoflavones

Compound	Initial weight g	Final weight g	Increment after 30 days	Average increment, g	% with respect to control	% with respect to diiodotyrosine (DIT)
I	830	1513	683	22,7	11,0	2,2
II	756	1638	882	29,4	13,2*	2,3*
III	780	1695	915	30,5	13,7*	2,8*
IV	924	1750	826	27,5	23,8*	11,5*
V	854	1481	627	20,9	1,4	—
VI	854	1524	679	22,6	9,7	1,8
VII	167	1142	975	32,5	9,8**	1,8**
VIII	760	1427	667	22,2	7,7	—
IX	138	1128	990	33,0	11,4**	3,4**
X	868	1540	672	22,4	8,7	0,9
Control	830	1448	618	20,6		
DIT	868	1524	666	22,2	7,7	—
* Control	818	1484	666	22,2		
DIT	700	1417	717	23,9	7,7	—
** Control	430	1318	888	29,6		
DIT	129	1085	956	31,9	7,7	—

TLC. The new isoflavones are colorless crystalline substances, which are readily soluble in organic solvents. The physical constants and spectral characteristics of the compounds obtained are given in Table 1.

The structure of the new compounds was confirmed by the data of quantitative elemental analysis, coinciding with the calculated values, and also by the PMR spectra. In contrast to the starting 2-hydroxydesoxybenzoins, in the spectra of the obtained isoflavones, the two-proton singlet of the methylene group and the weakfield peak of the phenol proton disappear, and instead a single sharp singlet of the 2-H pyrone ring proton appears in the 7.5-8.7 ppm region. Moreover, a characteristic indication of the formation of the chromone ring is the presence of a signal in the 8.2 ppm region, belonging to the aromatic 5H protons, which is descreened by the neighboring carbonyl group. These data enable a ready confirmation of the formation of an isoflavone system. In cinnamoyl derivatives of isoflavone, the spin-spin coupling constant for the olefin protons is 15 Hz on the average, which indicates a transoid configuration of the olefin fragment of the molecules of V and VI. In the PMR spectra of compounds IV, VII-X corresponding signals of the modified residues (alkoxy and ester groups) are also present.

The biological tests showed that, as was assumed, the isoflavones obtained have a notable anabolic effect, exceeding the effect of diiodotyrosine (DIT) occasionally used in veterinary practice as a growth promoter for agricultural animals [4]. The data in Table 3 show that modification of the isoflavone molecules by the introduction of fluorine atoms at the 2^1 and 4^1-positions and also by alkylation of the hydroxyl group at the 7-position, intensifies the effectiveness of the preparations.

It has been established experimentally that substitution of a hydrogen atom by fluorine in ring B of the isoflavone molecule leads to some decreases in toxicity (from 2000 to 2500 mg/kg). Introduction of an isopropyl fragment at the 7-position of the chromone ring has a noticeable effect on the decrease in toxicity (see Table 2). Introduction of one cinnamic acid residue into the isoflavone molecule leads not only to a decrease in the toxicity of the starting compound I, but also to a considerable decrease in the anabolic effect. However, increase in the specific weight of the isoflavonoid fragments bound to the cinnamic acid residue leads, besides to the decrease in toxicity, also to increase in the anabolic effect, compared with compound V. Alkylation of 7-hydroxisoflavone I by the α-propionic acid residue (in particular, by its methyl ester) does not alter the anabolic effect.

Thus, the biological investigations indicate a pronounced anabolic effect of compounds I-IV, VI-VII, IX-X, which can find application in medicine as a mammalian cell growth promoter and also in agriculture as a growth promoter of agricultural animals.

EXPERIMENTAL (CHEMICAL)

The course of the reactions and the purity of the compounds obtained were monitored by TLC on Silufol UV-254 plates (CSSR). A mixture of benzene and ethanol (95:5) or a mixture of chloroform and methanol (9:1) served as eluent. The PMR spectra were recorded on a WP-100SV spectrometer ("Bruker", GFR), with reference to TMS (internal standard). The IR spectra were run on a "Pye Unicam" spectrophotometer (England) in KBr tablets. The characteristics of compounds I-X are given in Table 1.

General Procedure for the Preparation of 7-Hydroxyisoflavones I-III. A 12 ml portion (100 mmoles) of boron trifluoride etherate was added to a solution of 50 mmoles of the corresponding 2,4-dihydroxydesoxybenzoin in 75 ml (100 mmoles) of DMFA, and immediately thereafter, 5.29 ml (60 mmoles) of phosphorus trichloride was added. After mixing of all the reagents, the mixture was held at 50-60°C, simultaneously collecting the ether that separated out. The reaction mixture was poured into 400 ml of water. The precipitate formed was filtered off and recrystallized from a suitable solvent (compound I from benzene, compound II and III from isopropanol).

General Procedure for the Preparation of 7-Isopropoxyisoflavones IV, VII, IX. A 6.8 g portion (40 mmoles) of isopropyl iodide was added dropwise to a mixture of 20 mmoles of the corresponding 7-hydroxyisoflavone in 50 ml of dry acetone in the presence of 8.3 g (60 mmoles) of calcined potassium carbonate. After mixing all the reagents, the temperature of the reaction mixture was held at 56°C. The end of the reaction was determined by TLC. The reaction mixture was cooled, the inorganic precipitate was separated by filtration, the solvent was evaporated on a rotary evaporator and the residue was recrystallized from acetone.

General Procedure for the Preparation of 7-(1-Methyl-1-methoxy-carbonyl)methoxyisoflavones VIII and X. A 4.2 g portion (30 mmoles) of a freshly calcined potassium carbonate and 2 g (12 mmoles) of methyl α-bromopropionate were added to a suspension of 10 mmoles of R^1-fluoro-7-hydroxyisoflavone in 60 ml of dry acetone, and the reaction mixture was allowed to stand for 2-3 h at 50-56°C (the end of the reaction was determined by TLC). After cooling the reaction mixture, the inorganic precipitate was filtered off, and the filtrate was evaporated under a water aspirator vacuum. The precipitate obtained was recrystallized from ethanol.

7-Cinnamoyloxyisoflavone (V). A 2.35 g portion (10 mmoles) of 7-hydroxyisoflavone I was dissolved in 50 ml of dry pyridine, and then a 1.8 g (11 mmoles) of freshly distilled cinnamoyl chloride was added and the mixture was boiled under a reflux condenser for 1.5 h without access to atmospheric moisture. After cooling, 200 ml of a 25% aqueous solution of acetic acid was added to the reaction mixture. The coagulated white precipitate was filtered off and recrystallized from toluene. Yield, 2.5 g. IR spectrum, ν, cm^{-1}: 1733 (C=O of the cinnamyl fragment), 1640 (C=O of chromone).

1,4-Di(β-propenoyloxyisoflavonyl-7)benzene (VI). A 1.74 g portion (12 mmoles) of para-phenylenediacryloyl dichloride in 12 ml of toluene was added to a solution of 4.7 g (20 mmoles) of 7-hydroxyisoflavone I in 50 ml of dry pyridine. The reaction mixture was boiled for 4 h without access to moisture. After cooling, the precipitate was filtered off and washed with 50 ml portions of a 25% aqueous solution of acetic acid (200 ml). The material was dried and recrystallized from dimethylformamide. Yield 4.9 g, IR spectrum, ν, cm^{-1}: 1720 (C=O of the propenoyloxybenzene fragment), 1660 (C=O of chromone).

EXPERIMENTAL (BIOLOGICAL)

The acute toxicity of compounds I-X studied was determined according to the method of B. M. Shtabskii et al., [6]. To accomplish this, the compounds studied were administered to two groups of 6 animals in doses of 2000 and 4000 mg/kg of body weight, respectively, of the animal. The calculations were carried out according to the formula:

$$X = \frac{(Y - Y_1)(X_2 - X_1)}{(Y_2 - Y_1)} + X_1,$$

where X_1 is the smaller dose; X_2 – larger dose; X – sought-for dose; Y_1 – percent of lethality at the smaller dose; Y_2 – at the larger dose; Y – sought-for percent of lethality (LD_{50}). It was found that the acute toxicity of the compounds studied is within 2000-3500 mg/kg (see Table 2).

The study of the growth promoting action of compounds I-X was carried out on chicks of the Leghorn genus, of a similar age, weight and origin. For the 30 days of the preparative period, the chicks were kept outdoors and were sustained on various feeds.

The feed ration included wetted milled wheat-oat mixture, freshly harvested green mass of corn at the stage of milky-waxy ripeness, a green mass of Lucerna, and carrots.

The compounds studied were administered to the chicks intravenously in the region of the pectoral muscle in a single dose of 100 mg/kg in 2.5 ml of a sterile solution of Trivit (a complex vitamin A, D, E preparation). The effectiveness of the tested compound was determined from the increase in weight gain of the chicks 30 days after the beginning of administration of the compound, without taking into account the latent period (5 days). From the data obtained, the increment in live weight of the chicks in the course of 30 days was calculated, compared with control – a solution of Trivit in a dose of 2.5 ml – and compared with diiodotyrosine in a dose of 100 mg/kg in an oily solution of Trivit.

The results of the biological tests were processed statistically; they are presented in Table 3. Before introducing the tested compounds, the chicks were weighed several times during the preparative period, which made it possible at the moment of the administration of the compound to select a group of 10 chicks with a similar rate of natural growth. In all the experiments, $p < 0.001$.

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