SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 21-ESTERS OF PREDNISOLONE WITH GLYCINE AND GLUTAMIC ACID

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Recently there have been studies on condensing endogenous corticosteroids and their analogs with various natural and synthetic amino acids, with the aim of developing biologically active compounds with novel properties [1, 2, 4-6]. Thus we know that 21-esters of corticosteroids with methionine and other sulfur-containing amino acids, with the terminal amino groups protected by acetyl or carbobenzoxyl groups, possess anti-inflammatory properties [6]. Esters of cortisol and prednisolone with N-(2-chloroethyl)-N-nitrosocarbamoyl derivatives of amino acids (alanine and others) have been obtained which inhibit tumor growth [2]. Acetylation products of the 21-hydroxyl group of prednisolone and triamcinolone with 4-N-acetylaminomethylcyclohexane carboxylic acid 4-chloroanhydride possess antianaphylactic and anti-inflammatory activities [1].

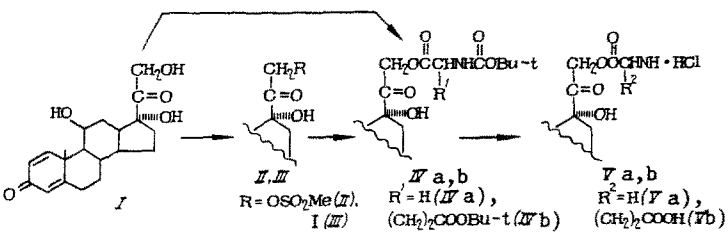
In the literature are also described 21-esters of corticosteroids with dicarboxylic acids linked to peptides made up of natural amino acids (glycine, alanine, methionine, etc.) [4]. Their sodium salts are used as water-soluble therapeutic agents, which are hydrolyzed in vivo to the original active preparations.

Comparatively recently we have become aware of 21-complex esters of corticosteroids with glycine and glutamic acid, having either free or substituted amino groups [5]. These derivatives are not water-soluble. Furthermore, the authors of the patent [5] did not supply data on the biological activity of these compounds, only speculating on the possibility of their use as drugs for local application.

It is well known [3] that corticosteroids show anti-inflammatory, anti-allergic, and anti-shock activities. In this connection, the development of water-soluble derivatives of corticosteroids, suitable for administration by parenteral injection, eyedrops, inhalation, etc. is important.

We have synthesized the hydrochlorides of 21-aminoacetoxy and 21-α-L-glutamyloxy-11β, 17α-dihydroxypregna-1,4-diene-3,20-dione (Va and b), which are soluble in water, and carried out a comparative study of their pharmacological properties, in order to determine the influence of an amino acid residue on some of the effects of a glucocorticosteroid (prednisolone).

Hydrochlorides Va and Vb were synthesized as in [5], by reacting the corresponding iodo derivative (III), obtained from the 21-dihydroxysteroid (I) via the 21-mesylate (II), with the potassium salts of amino acids that have the terminal amino group protected with the tert-butyloxycarbonyl (Boc) function. Another method for synthesizing 21-esters of prenisolone with amino acids, consisting of the direct reaction of the corticosteroid I with the anhydride of the Boc-protected amino acid, was found to be unsatisfactory, due to the low yield of the desired product and the impossibility of completely purifying it from dicyclohexylurea.



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In the case of glutamic acid, in order to selectively carry out the reaction with the α-carboxyl group, the γ-carboxyl group was protected in the form of its tert-butyl ester, the hydrolysis of which was accomplished at the same time as the removal of the Boc group by the action of a solution of HCl in dioxane [7]. This resulted in the protonation of the terminal amino group, with the formation of the water-soluble hydrochlorides Va and Vb in yields of 81.6 and 75%, respectively (calculated on starting prednisolone I).

It should be emphasized that in the course of the hydrolysis of the protecting groups (pH 1-2), the formation of the products of acid-catalyzed dienone-phenol rearrangement, occurring in the aromatic A-ring of the steroid molecule, was not observed, as shown by PMR and UV spectroscopy. In the PMR spectra of the obtained hydrochlorides Va and Vb, the signal corresponding to the proton at C-4 at "δ" 6.01 ppm was broad, due to spin-spin coupling with protons in positions 2 and 6. The protons at C-2 were present in the region of 6.25 ppm as a quartet (J_{12} 8.2 Hz, J_{24} 2 Hz). The resonance signals of the C-1 protons were displayed at weaker field, as a doublet (J 10 Hz) centered at "δ" 7.48 ppm.

The presence in the UV spectra of absorption at 243 and 244 nm respectively also confirms the preservation of the dienone system in the A-ring.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a Perkin–Elmer 599 instrument (Sweden), PMR spectra on a Varian X-200 (Germany), and mass spectra on a Varian MAT-112 (Germany) with an ionization energy of 50 eV. TLC was done on Silufol-254 plates in the systems $CHCl_3$–acetone–cyclohexane, 6:3:1, and $CHCl_3$–MeOH–ammonia, 9:1:0.5. Values found in elemental analysis agreed with those calculated.

11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione 21-Mesylate(II). To a solution of 1.5 g (4.17 mmoles) of prednisolone (I) in 10 ml pyridine was added at 0°C 1.5 ml (16.5 mmoles) methane sulfochloride. After 30 min the reaction was poured into 100 ml of ice water, filtered, washed with water, and dried. Obtained: 1.81 g (99.2%) compound II, mp 200-202°C (dec.), lit. [8] mp 206°C (dec.). IR spectrum, ν_{max}, cm^{-1}: 3540 and 3300 (OH) 1730 [C(20)=O], 1660 [C(30)=O], 1610 and 1600 (C=C)]. Mass spectrum, m/z: [438 (M^+), 420, 342, 324, 306, 265 (100%)].

11β,17α-Dihydroxy-21-iodopregna-1,4-diene-3,20-dione (III). To a solution of 1.81 g (4.13 mmoles) compound II in 90 ml acetone was added a solution of 1.81 g (12.06 mmoles) NaI in 25 ml of acetone. This was boiled 15 min and evaporated. The residue was transferred to Na_2SO_4 solution, filtered, washed with water, and dried. Obtained: 1.81 g (93.2%) compound III, mp 185-187°C (dec.), lit. [8] mp 188°C (dec.). IR spectrum, ν_{max}, cm^{-1}: 3400 and 3280 (OH), 1710 [C(10)=O], 1650 [C(3)=O], 1600 and 1590 (C=C), 590 (C–I)]. Mass spectrum, m/z: [470 (M^+). 343. 325, 265, 253 (100%)].

21-(N-tert-Butyloxycarbonyl)aminoacetoxy-11β,17α-dihydroxypregna-1,4-diene-3,20-dione (IV). To a solution of 2.18 g (4.63 mmoles) of the iodo derivative of III in 12 ml DMFA was added a solution of 1.21 g (5.67 mmoles) of the potassium salt of Boc-glycine in 10 ml DMFA (obtained by dissolving 1 g Boc-glycine and 0.32 g KOH in 4 ml water and evaporating with dioxane). After 4 h the reaction mass was poured into 400 ml ice water, and the residue was filtered, washed with water, and dried. Obtained: 2.33 g (97%) compound IVa, mp 102-104°C. PMR spectrum, $CDCl_3$, δ, ppm: 0.95 s (3H, 18-CH_3), 1.45 s [3H, 19-CH_3, 9H, $C(CH_3)_3$], 4.05 d (2H, N-CH_2, J = 5.72 Hz), 4.49 br. s (1H, 11-H), 5.01, centered AB system (2H, 21-CH_2, J = 17.5 Hz), 5.11 (1H, NH, J = 3 Hz), 6.01 s (1H, 4-H), 6.27 dd (1H, 2-H, J_1 = 10 Hz, J_2 = 1 Hz), 7.33 d (1H, 1-H, J = 10 Hz).

21-Aminoacetoxy-11β,17α-dihydroxypregna-1,4-diene-3,20-dione Hydrochloride (Va). To a suspension of 3.28 g (6.34 mmoles) compound IVa in 7 ml CH_2Cl_2 was added 15 ml (45 mmoles) of a 3 N solution of HCl in dioxane. After 2.5 h the reaction mass was evaporated and 20 ml of acetone was added. The residue was filtered, washed with acetone, and dried in a desiccator over alkali. Obtained: 2.61 g (91%) compound Va in the form of white crystals. Yield 81.6% calculated on prednisone (I). mp 210-212°C. $C_{23}H_{32}ClNO$. IR spectrum, ν_{max}, cm^{-1}: 3320 (OH), 3080, 2700 and 2610 (hydrochoride), 1780 (COOR), 1720 [C(20)=O], 1660 [C(3)=O], 1610 and 1600 (C=C), 1520 (amine II), 1220 (C–O). PMR spectrum, CD_3OD, δ, ppm: 0.91 s (3H, 18-CH_3), 1.48 s (3H, 19-CH_3), 4.00 s (2H, N-CH_2), 4.42 d (1H, 11-H, J = 2.7 Hz), 5.16 centered AB system (2H, 21-CH_2, J = 17.5 Hz), 6.01 br. s (1H, 4-H), 6.25 dd (1H, 2-H, J_1 = 8 Hz), 7.47 d (1H, 1-H, J = 10 Hz). UV spectrum, λ_{max} (C_2H_5OH): 243 nm (ε 15,000).

TABLE 1. Anti-Inflammatory Activity and Systemic Effects of Prednisolone Analogues

Compound	Weight of dry granulomas, mg	Thymus weight, g	Increase in body weight, g	Weight of adrenals, mg	Plasma corticosterone, μg
Control	29,6±1,6	0,37±0,02	41,3±2,1	18,0±0,6	32,9±1,4
Va	18,3±1,0*	0,30±0,03*	28,7±5,0*	17,7±0,9	28,7±2,2
Vb	20,4±1,1*	0,27±0,02*	29,8±3,0*	16,2±0,5	27,4±3,0
Prednisolone hemisuccinate	22,5±2,1*	0,24±0,02*	32,2±4,2*	16,6±0,7	24,3±1,7*

*At p = 0.05, difference from control statistically significant.

21-(N-tert-Butyloxycarbonyl)-(γ-tert-butyl ester)-α-L-glutamyloxy-11β,17α-dihydroxypregna-1,4-diene-3,20-dione (IVb). Under the conditions used to obtain compound IVa, from 1.81 g (3.84 mmoles) iodo derivative III reacting with 1.7 g (4.97 mmoles) of the potassium salt of the γ-tert-butyl ester of Boc-L-glutamic acid (a solution of 1.52 g of the γ-tert-butyl ester of Boc-L-glutamic acid and 0.28 g KOH in 15 ml of water was evaporated under vacuum with dioxane) we obtained 2.2 g (88.7 g) of IVb, mp 107-110°C. IR spectrum, $CHCl_3$, ν_{max}, cm^{-1}: 3608 (OH), 3440 (NH), 1730 (COOR), 1715 [C(20)=O], 1660 [C(3)=O] (amide I), 1620 and 1600 (C=C), 1500 (amide II), 1155 (C–O). PMR spectrum, $CDCl_3$, δ, ppm: 0.94 s (3H, 18-CH_3), 1.43 s (9H, γ-tert-butyl ester), 1.45 s (3H, 19-CH_3; 9H, Boc) 4.37 m (1H, NCH), 4.49 br. s (1H, 11-H), 4.90 entered AB system (2H, 21-CH_2, J = 17 Hz), 5.27 d (1H, NH, J = 8.4 Hz), 6.01 s (1H, 4-H), 6.27 dd (1H, 2-H, J_1 = 1.8 Hz), 7.32 d (1H, 1-H, J = 10.2 Hz).

An analytical sample was obtained by chromatography on a column of silica gel (40 × 100), with $CHCl_3$ as eluent. mp 112-114°C, $C_{35}H_{51}NO_{10}$.

21-α-L-Glutamyloxy 11β,17α-hydroxypregna-1,4-diene-3,20-dione Hydrochoride (Vb). Under the conditions used to obtain compound Va, from 1 g (1.55 mmoles) IVb and 5 ml of 3 N HCl in dioxane (14.97 mmoles) we obtain 0.8 g (98%) compound Vb as white crystals. mp 139-140°C. After recrystallization from MeOH-ether, 1:8, we obtain 0.75 g (91.8% Vb. Yield 75% based on prednisolone I). mp 141-143°C. $C_{26}H_{36}ClNO_8$. IR spectrum, ν_{max}, cm^{-1}: 3380 (OH), 3070, 2710, 2630 (hydrochloride), 1750 (COOR), 1720 br. [COOH, C(20)=O], 1660 [C(3)=O) 1600 (C=C), 1500 (amine II), 1225 (C–O)]. UV spectrum, λ_{max} (C_2H_5OH): 244 nm (ε 15,200). PMR spectrum, CD_3OD, δ, ppm: 0.91 s (3H, 18-CH_3), 1.49 s (3H, 19-CH_3), 4.27 t (1H, NCH, J = 6.2 Hz), 4.43 d (1H, 11-H, J = 3 Hz), 5.17 centered AB system (2H, 21-CH_2, J = 18 Hz), 6.01 s (1H, 4-H), 6.25 dd (1H, 2-H, J_1 = 8.2 Hz, J_2 = 1.9 Hz), 7.48 d (1H, 1-H, J = 10 Hz).

EXPERIMENTAL (PHARMACOLOGICAL)

The purpose of the pharmacological investigation was to determine the pharmacological activities and systemic effects of the compounds synthesized. With this aim we studied the effects of Va and Vb on acute (exudative) and chronic (proliferative) inflammatory reactions, and their antishock activity, compared to prednisolone hemisuccinate. The compounds under investigation and prednisolone hemisuccinate were given in the form of aqueous solutions.

Acute exudative inflammation was brought about in rats of both sexes weighing 140-160 g by subplantar injection (into the left hind extremity) of 0.1 ml of a 1% carrageenan suspension in isotonic saline. Swelling was measured plethysmometrically 3 h after injection of the carrageenan, and was expressed as the difference between the volumes of the right and left feet. The test compounds were injected subcutaneously in a dose of 5 mg/kg, 1 h prior to the injection of carrageenan [10].

Chronic proliferative inflammation was induced by subcutaneous implantation of two sterile felt balls in male rats weighing 80-100 g. Test compounds were injected subcutaneously in a dose of 5 mg/kg per day, starting on the day the felt was implanted. After seven days the animals were sacrificed, and the weights of the dry granulomas, thymus, and adrenals were determined, as well as the increase in body weight and content of corticosterone in blood plasma [9, 11].

Antishock activity of the compounds was tested in adrenalectomized male rats weighing 100-120 g. Shock was induced by injection into a tail vein of 1 ml of polyglucin (a 6% dextran solution) which caused the death of the animals over the next 24 h. Compounds were given subcutaneously in a dose of 5 mg/kg, 4 h prior to the injection of polyglucin.

The experiments showed that the compounds synthesized did not differ from prednisolone hemisuccinate in their effects on the exudative reaction; when injected in a dose of 5 mg/kg, they reduced carrageenan-induced swelling by 40-55%.

The effects on chronic proliferative inflammation, along with the systemic effects of the synthesized compounds and the reference preparation, are given in Table 1. It is evident that with daily injection in a dose of 5 mg/kg for 7 days, all compounds studied inhibited the formation of granulomatous tissue by 25-30%. Prednisolone hemisuccinate and Vb were found to have the greatest thymolytic activity (35 and 27%, respectively); Va decreased the mass of the thymus by 19%. Compounds Va and Vb and the reference preparation caused the animals to gain weight to a similar degree, but did not decrease the weight of the adrenals. At the same time, it was found that the compounds synthesized did not change the content of corticosterone in blood plasma, in contrast to prednisolone hemisuccinate, subcutaneous administration of which changed the hormone's concentration by 26%.

in terms of antishock activity, both compounds and the reference preparation did not differ; at a subcutaneous dose of 5 mg/kg, they resulted in a survival rate of 50-70%, while in the control group, all the animals died.

Thus, the incorporation of amino acid residues into a molecule of prednisolone hemisuccinate enables one to obtain soluble compounds which, in their spectrum of phrmacological activity (reduction in exudative and proliferative inflammatory reactions and antishock activity), do not differ from prednisolone hemisuccinate; at the same time, they cause less pronounced systemic side effects when given by subcutaneous injection for 7 days.

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SYNTHESIS AND ANTIVIRAL ACTIVITIES OF 5-(PYRIDYL-2)-OXYINDOLE DERIVATIVES

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Among the derivatives of phenoxybenzoles and phenoxypyridines are found compounds which exhibit antiviral activities [7]. During their studies of structure-activity relationships, the authors of the referenced work concluded that one or more electron-accepting substituents must be present on the aromatic or heteroaromatic (pyridine) ring for the compound to be biologically active. Although the biochemical causes for the antiviral activity of this class of compound are not fully understood, the authors proposed that the inhibition of nucleic acid synthesis plays a role, and that the activities of these electron-deficient aromatic (and heteroaromatic) ethers may be due to their possible capabilities to form σ-complexes with nucleophilic regions of virus proteins [7].

With the above as a starting point, we sought to synthesize heteroatomic ethers with pyridine and indole rings, especially since 5-oxyindole derivatives have been found which

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