

Synthesis and Local Anti-inflammatory Activity of Esters of 20R- and 20S-Dihydroprednisolonic Acid

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Abstract □ The various esters of 20R and 20S-dihydroprednisolonic acid were prepared and evaluated for their local and topical anti-inflammatory activities in rats, including systemic effects. It was demonstrated that the size of the substituent at C-21 and configurational arrangement at C-20 have significant effects on local and topical activity. It was also found that all the derivatives did not show systemic effects as assessed by thymus weight and adrenal weight at the dose level used, while prednisolone showed profound effects on thymus weight.

Keywords □ Esters of 20-dihydroprednisolonic acid, anti-inflammatory activity, systemic effects

For developing local anti-inflammatory steroids with reduced systemic side-effects, various synthetic modifications were carried out¹⁻⁵. The rationale for the modification is preparing derivatives which show local activity at the applied area, but rapidly convert to inactive compounds in the systemic circulation, giving reduced systemic side-effects. On the basis of this idea, methyl 20R and 20S-dihydroprednisolone were prepared from prednisolone. Of these compounds, the methyl 20S-isomer showed considerable local anti-inflammatory activity without systemic side-effects in rat cotton pellet granuloma bioassay^{1,2}. Using various primary amides, 20R and 20S-dihydro-20-carboxamides of prednisolone were synthesized from each 20R and 20S-dihydroprednisolonic acid. In contrast to the methyl ester derivatives, 20R isomers of these carboxamide series were always found to possess higher local anti-inflammatory activity than the corresponding isomers³. Recently, introduction of a methoxycarbonyl group at the C-16 α position resulted in the compound having 5.5 times more local activity with significantly reduced systemic side-effects than prednisolone. In addition, there was a

dramatic increase in topical activity in the croton-oil induced rat ear-edema bioassay^{4,5}.

In this report, various ester derivatives of 20R and 20S-dihydroprednisolonic acid were prepared and anti-inflammatory activities were compared in order to study the effects of the different substituents at C-21 and C-20 configuration. The effects of substituents of these ester derivatives on the hydrolysis to inactive acid derivatives are presented in the accompanying paper.

EXPERIMENTAL METHODS

Materials

Prednisolone was purchased from Sigma Chem. Co. (St. Louis, MO). Melting points were determined on a Thomas capillary melting point apparatus and are uncorrected. ¹H-NMR spectra were obtained with Varian FT-80 MHz NMR and chemical shifts were reported in part per million (δ). Optical rotation was measured with Rudolph Autopol III. The HPLC system (Waters Associates) consisted of a C-18 reverse-phase analytical cartridge (Merck) or semi-preparative C-8 Lobar column (0.5 \times 10 cm, Merck) and the detector wavelength was set at 254 nm. The flow rate was 1.0 ml/min for analytical se-

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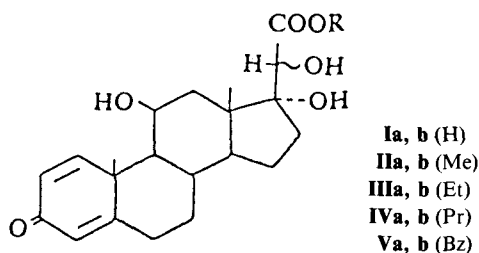


Fig. 1. Derivatives of prednisolone.

(a denotes 20R-isomer, b denotes 20S-isomer)

paration with MeOH/water (60:40) as a mobile phase. Silica gel (70-230 mesh, Merck) was used for column chromatographic separation. The homogeneity of the intermediates and products was determined by HPLC and TLC. Spots on TLC (60F-254) were visualized under UV light. Elemental analyses were performed by Analytical Lab. at KAIST (Korea).

Synthesis of ester derivatives (Fig. 1)

Using prednisolone as a starting material, each isomer of methyl 20-dihydroprednisolonate (**IIa,b**) was obtained according to the method of Kim *et al.*³⁾ From the respective isomer of **IIa,b**, ethyl (**IIIa,b**) and propyl (**IVa,b**) esters were synthesized with conventional transesterification reactions using alcoholic NaOH solution. Following the reaction at room temp. for 1-4 hrs, the reaction mixture was neutralized with 1 N HCl and diluted with water. The solution was extracted with ethyl acetate and the organics were dried over anhydrous sodium sulfate. After evaporating the organics, the crude mixtures were applied on silica gel column (60 × 2.5 cm) and eluted using the solvent mixture, acetone/dichloromethane/hexane (3:2:5), except for ethyl 20S-dihydroprednisolonate (**IIIb**) using acetone/dichloromethane/hexane (2:2:5). The fractions corresponding to each compound were combined and evaporated. Ethyl 20S-dihydroprednisolonate, little contaminated with **IIIb** was further purified with semi-preparative HPLC using a C-8 Lobar column. The mobile phase (50% EtOH) was used with the flow rate of 0.5 ml/min. Retention time was found to be approximately 1 hr. The combined fraction was evaporated to give **IIIb** as a white foam. From each isomer of 20R and 20S-dihydroprednisolonic acid (**Ia,b**) prepared as described previously³⁾, benzyl ester derivatives (**Va,b**) were synthesized with esterification reaction. Briefly, benzyl alcohol (4 ml) and three drops of concentrated-

sulfuric acid were added to a solution of **Ia** (200 mg) in dichloromethane and refluxed for 2 hrs. After evaporating the solvent, the mixture was purified with silica gel column (80 × 2.5 cm) using acetone/dichloromethane/hexane (2:2:5). Recrystallization from acetone gave pure **Va** (110 mg). Benzyl 20S-dihydroprednisolonate (**Vb**) was prepared from **Ib** using the same procedure as described in the synthesis of **Va**. The physical properties of the ester derivatives were shown in Table I.

Ethyl (20R)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregna-21-oate (**IIIa**)

¹H-NMR (CDCl₃) δ 1.15(s, 3H, 13-CH₃), 1.35(t, 3H, J = 8 Hz, 21-CH₃), 1.45(s, 3H, 10-CH₃), 4.1-4.3(m, 3H, 20-H and 21-OCH₂-), 4.40(m, 1H, 11-H), 6.0(s, 1H, 4-H), 6.20(dd, 1H, J = 10 and 2 Hz, 2-H), 7.15(d, 1H, J = 10 Hz, 1-H). Anal. Calcd for C₂₃H₃₂O₆: C, 68.29; H, 7.97. Found: C, 68.66; H, 7.90.

Ethyl (20S)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregna-21-oate (**IIIb**)

¹H-NMR (CDCl₃) δ 1.15(s, 3H, 13-CH₃), 1.30(t, 3H, J = 8 Hz, 21-CH₃), 1.45(s, 3H, 10-CH₃), 4.10-4.40(m, 4H, 11-H, 20-H and 21-OCH₂-), 5.95(s, 1H, 4-H), 6.20(dd, 1H, J = 10 and 2 Hz, 2-H), 7.20(d, 1H, J = 10 Hz, 1-H). Anal. Found: C, 67.40; H, 7.43.

n-Propyl (20R)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregna-21-oate (**IVa**)

¹H-NMR (CDCl₃) δ 1.0(t, 3H, J = 8 Hz, 21-CH₃), 1.10(s, 3H, 13-CH₃), 1.40(s, 3H, 10-CH₃), 4.0-4.25(m, 3H, 20-H and 21-OCH₂-), 4.40(m, 1H, 11-H), 5.95(s, 1H, 4-H), 6.20(dd, 1H, J = 10 and 2 Hz, 2-H), 7.15(d, 1H, J = 10 Hz, 1-H). Anal. Calcd for C₂₄H₃₄O₆: C, 68.88; H, 8.19. Found: C, 69.11; H, 8.28.

n-Propyl (20S)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregna-21-oate (**IVb**)

¹H-NMR (CDCl₃) δ 0.95(t, 3H, J = 8 Hz, 21-CH₃), 1.15(s, 3H, 13-CH₃), 1.40(s, 3H, 10-CH₃), 4.1(t, 2H, J = 6 Hz, 21-OCH₂-), 4.2-4.45(m, 2H, 11-H and 20-H), 5.95(s, 1H, 4-H), 6.20(dd, 1H, J = 10 and 2 Hz, 2-H), 7.20(d, 1H, J = 10 Hz, 2-H). Anal. Found: C, 67.11; H, 8.47.

Benzyl (20R)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregna-21-oate (**Va**)

¹H-NMR (CDCl₃) δ 0.95(s, 3H, 13-CH₃), 1.35(s,

3H, 10-CH₃), 3.95-4.20(m, 2H, 11-H and 20-H), 5.2(m, 2H, 21-OCH₂-), 5.90(s, 1H, 4-H), 6.15(dd, 1H, J = 10 and 2 Hz, 2-H), 7.10(d, 1H, J = 10 Hz, 1-H), 7.35(s, 5H, 21-C₆H₅). Anal. Calcd for C₂₈H₃₄O₆: C, 72.08; H, 7.34. Found: C, 71.84; H, 7.44.

Benzyl (20S)-11 β ,17,20-trihydroxy-3-oxo-1,4-pregnadien-21-oate (Vb)

¹H-NMR (CDCl₃) δ 1.10(s, 3H, 13-CH₃), 1.40(s, 3H, 10-CH₃), 4.25-4.45(m, 2H, 11-H and 20-H), 5.15(m, 2H, 21-OCH₂-), 5.95(s, 1H, 4-H), 6.15(dd, 1H, J = 10 and 2 Hz, 2-H), 7.15(d, 1H, J = 10 Hz, 1-H), 7.30(s, 5H, 21-C₆H₅). Anal. Found: C, 71.80; H, 7.31.

Evaluation of anti-inflammatory activity

For measuring local anti-inflammatory activity, he slightly modified rat cotton pellet bioassay was used.³⁾ Male Sprague-Dawley rats (100-150 g) were maintained in separate cages with Purina Lab Chow (Purina, Korea) and given tap water *ad libitum*. The animal quarters were maintained at 21-24°C and 40-50% relative humidity. A 12 hr L/D cycle was used. Prior to implantation of cotton pellets (35 \pm 1 mg, Richmond Dental Co., Charlotte, NC), 2.5 mg of steroid derivatives, dissolved in acetone, were applied to the treated pellet while the untreated pellet received an equal volume of vehicle. Two untreated pellets were implanted in control animals, and one treated (right) pellet and one untreated (left) pellet were implanted in test animals. Animals were sacrificed 7 days later, and cotton pellets were recovered, dried at 60°C for 2 days and weighed. Thymus tissue and adrenal gland weights were recorded for the evaluation of systemic side-effects.

For comparing topical anti-inflammatory activity, the croton-oil induced rat ear-edema assay of Bird *et al.*²⁾ was used. Male Sprague-Dawley rats (100-120 g) were habituated to measuring ear thickness with a micrometer (0.01 mm unit, Fowler Precision Tools) for 2 days prior to experiment. On the day of experiment, 25 μ l of vehicle (acetone) or drug solution was applied and 30 min later, 25 μ l/ear of 5% croton oil in acetone was applied. After 5 hrs, ear thickness was measured and dose-response curves were constructed using percent inhibition of ear thickness.

RESULTS

The physical properties of the synthesized derivatives are summarized in Table I. In synthesis of

Table I. Physical data of the derivatives

Compounds	m.p.(°C)	$[\alpha]_D^{20}$ ^a	Rf(TLC) ^b	RT(min) ^c
IIIa	214.5-217 ^d	+46.6	0.47	17.19
IIIb	amorphous	+1.5	0.43	30.34
IVa	200-202.5 ^e	+50.3	0.54	24.80
IVb	amorphous	-6.5	0.47	52.75
Va	180.5-183 ^e	+60.6	0.66	39.70
Vb	198-201 ^e	-16.9	0.56	79.75

^aApproximately 1% solution (MeOH), ^bchloroform/MeOH (9:1), ^cRetention time in HPLC, ^dRecrystallized from ethyl acetate, ^eFrom acetone.

benzyl derivatives, the conventional esterification method was employed (over 70% yield) because the yield using transesterification was less than 15%. In optical rotation, all 20R-isomer showed more dextrorotation than the respective 20S-isomer. Table II illustrates the local anti-inflammatory activities and systemic effects of the derivatives at the dose of 2.5 mg/pellet. While prednisolone significantly inhibited granuloma formation, 71% in the treated side and 43% in the untreated side, all the derivatives were shown to possess less local activity. At this dose, the order of activity was prednisolone > propyl 20S-dihydroprednisolonate = methyl 20S-dihy-

Table II. Effects of the derivatives on cotton pellet granuloma formation

Compd's ^a	Treated side		Untreated side		Thymus wt. (mg/100 g body wt.) ^b
	Granuloma wt. (mg)	Inhibition(%)	Granuloma wt. (mg)	Inhibition(%)	
Control	60 \pm 4.5 ^c		55 \pm 3.6		344 \pm 25
Prednis.	18 \pm 0.8**	71	31 \pm 3.3*	43	154 \pm 11**
IIa	52 \pm 6.6	13	56 \pm 2.8		331 \pm 19
IIb	33 \pm 2.2**	45	51 \pm 1.7	7	284 \pm 17
IIIa	39 \pm 3.7*	35	44 \pm 4.5	20	296 \pm 11
IIIb	41 \pm 3.3*	32	45 \pm 2.9	17	267 \pm 14
IVa	41 \pm 3.3*	32	50 \pm 4.5	9	349 \pm 19
IVb	33 \pm 2.0**	45	40 \pm 1.6*	27	307 \pm 21
Va	41 \pm 3.7*	32	45 \pm 4.1	17	292 \pm 7
Vb	40 \pm 3.3*	42	47 \pm 2.0	14	296 \pm 29

^aAll the steroid derivatives were loaded at the treated side (2.5 mg/pellet).

^badrenal wt. was not shown here, but no significant difference was observed in all derivatives.

^cData represents mean \pm SE of six animals. Statistical significance vs control assessed by one way ANOVA, p > 0.001 (**), p > 0.01 (*).

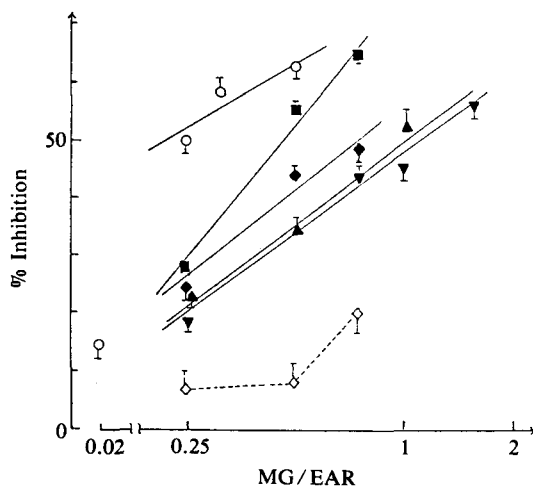


Fig. 2. Topical anti-inflammatory activity of the derivatives in ear edema bioassay. prednisolone (○), IIB (▲), IIIb (▼), IVb (■), Vb (◆), Va (◇).

droprednisolonate. The other derivatives showed much lower anti-inflammatory activity. In general, the 20S-isomers showed higher activity than the respective 20R-isomers except for the ethyl ester derivatives. Prednisolone reduced thymus weight dramatically but did not alter adrenal weight. None of the derivatives had effects on these parameters. The results of ear-edema bioassay are presented in Fig. 2. The order of potency are prednisolone > propyl 20S-dihydroprednisolonate > benzyl 20S-dihydroprednisolonate > methyl 20S-dihydroprednisolonate > ethyl 20S-dihydroprednisolonate. All of 20R-isomers showed much lower activities (data not shown). The ID_{50} values are 0.23 mg/ear for prednisolone, 0.46 mg for propyl 20S-isomer, 0.76 mg for benzyl 20S-isomer, 0.97 mg for methyl 20S-isomer and 1.14 mg for ethyl 20S-isomer.

DISCUSSION

In this communication, we have prepared methyl, ethyl, n-propyl and benzyl ester derivatives of 20R and 20S-dihydroprednisolonic acid, in order to investigate the structural-activity relationship at C-21 and C-20 of a series of the derivatives. To evaluate the anti-inflammatory activity, rat cotton pellet granuloma bioassay and croton-oil induced ear edema method were employed. The local and topical anti-inflammatory activity expressed by methyl 20R and 20S-dihydroprednisolonate were well

matched with the previously published results^{1,2}. The potency of all of these ester derivatives, related to prednisolone, was lower, with the most active being two times less active in ear-edema bioassay. But it is interesting to note that stereochemical configuration at C-20 of these compounds is very important in eliciting higher biological activity. In contrast to carboxamide series³, 20S-isomer in the ester derivatives showed higher anti-inflammatory activity than 20R-isomer, especially in croton-oil induced ear-edema bioassay. It may be thought that spatial arrangement of the functional group around C-20 in the ester derivatives differs from that of carboxamides, even though 20R-isomer always showed higher binding affinity to the rat liver glucocorticoid receptor⁶. Another fact to be considered from this study is the size of the substituent of the derivatives at C-21. As many authors published data that the increased hydrophobicity would increase topical biological activity due to the rapid absorption through skin barrier^{7,8}, the derivatives having large hydrophobic substituents at C-21, propyl 20S and benzyl 20S-dihydroprednisolonate, possess the higher topical activity than those with small substituents. These results are well correlated with the 1-octanol/water partition coefficient of the derivatives⁹.

CONCLUSION

The size of the substituents at C-21 and configurational arrangement of C-20 have significant effects on local and topical anti-inflammatory activity of the ester derivatives. And these factors should be considered in the development of new local anti-inflammatory steroid derivatives in this series.

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LITERATURE CITED

1. Lee, H.J. and Soliman, M.R.I.: Anti-inflammatory steroid without pituitary-adrenal suppression. *Science* **215**, 989 (1982).
2. Bird, J., Kim, H.P. and Lee, H.J.: Topical anti-inflammatory activity of esters of steroid-21-oic acids. *Steroids* **47**, 35 (1986).
3. Kim, H.P., Bird, J., Heiman, A.S., Hudson, G.F., Teraporewala, I.B. and Lee, H.J.: Syn-

- thesis of new anti-inflammatory steroidal 20-carboxamides: (20R)- and (20S)-21-(N-substituted amino)-11 β ,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene. *J. Med. Chem.* **30**, 2239 (1987).
4. Taraporewala, I.B., Kim, H.P., Heiman, A.S. and Lee, H.J.: A novel class of anti-inflammatory steroids: I. Analogues of methyl 11 β ,17-21-trihydroxy-3,20-dioxo-pregna-1,4-diene-16 α -carboxylate. *Arzneim.-Forsch./Drug Res.* **39**, 21 (1989)
 5. Heiman, A.S., Kim, H.P., Taraporewala, I.B. and Lee, H.J.: A novel class of anti-inflammatory steroids: II. Pharmacological studies of methyl 11 β ,17,21-trihydroxy-3,20-dioxo-pregna-1,4-diene-16 α -carboxylate and methyl 11 β ,21-dihydroxy-3,20-dioxo-pregna-1,4-diene-16 α -carboxylate. *Arzneim.-Forsch./Drug Res.* **39**, 262 (1989).
 6. Kim, H.P., Lee, J.W., Kim, H.J., Byun, S.M. and Lee, H.J.: Development and structural-activity relationship of new local anti-inflammatory steroid, prednisolone derivatives (I). *Arch. Pharm. Res.* **10**, 184 (1987).
 7. Ponec, M., Kempenaar, J., Shroot, B. and Caren, J.-C.: Glucocorticoids: Binding affinity and lipophilicity. *J. Pharm. Sci.* **75**, 973 (1986).
 8. Sugai, S., Okazaki, T., Kajiwara, Y., Kanbara, T., Naito, Y., Yoshida, S., Akaboshi, S., Ikekami, S. and Kamano, Y.: Studies on topical anti-inflammatory corticosteroids (I). *Chem. Pharm. Bull.* **33**, 1889 (1985).