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Improved delivery through biological membranes. XII. The effect of the incorporation of biphasic solubilizing groups into prodrugs of steroids

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Summary

The functional groups contained in two penetration enhancers (dimethylsulfoxide and dimethylformamide) have been incorporated into the prodrug derivatives of steroids. These derivatives have the potential for increasing the biphasic solubility of the steroid and hence its rate of diffusion through skin. Most of the prodrugs synthesized were succinamate derivatives of hydrocortisone or hydrocortisone-17α-esters. Hydrocortisone-21-diethylsuccinamate (XVI) and hydrocortisone-21-diethylsuccinamate 17α-valerate (XXVI) were the most interesting prodrugs developed. The former derivative (XVI) almost doubled the rate of delivery of hydrocortisone through hairless mouse skin while at the same time it caused significantly less local toxicity than hydrocortisone. The latter derivatives (XXVI), on the other hand, caused significantly less systemic toxicity than either hydrocortisone or its parent 17α-ester. Both derivatives were more active than hydrocortisone-21-acetate.

Introduction

Since human skin is considered to be relatively lipoidal in nature, relatively polar drugs such as hydrocortisone do not easily penetrate it. Theoretically, one way to improve the topical penetration of hydrocortisone and similar glucocorticoid drugs would be to use penetration enhancers in their formulations. Here, a penetration

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enhancer is considered to be any chemical that is added to a formulation that increases the diffusion of the drug across a barrier — in this case the skin. Notable among the chemicals that have been shown to increase diffusion of drugs across the skin are dimethylsulfoxide and dimethylformamide (Maibach, 1976; Munro, 1969), as well as various amines (Allenby et al., 1969a). However, irritant action, odor and potential toxicity have prevented the widespread use of such penetration enhancers in topical formulations (Munro, 1969).

Thus, although there has been considerable work done to rationally vary the constituents of various topical formulations (Ostrenga et al., 1971) to improve the delivery of drugs (steroids) through the skin, there have not been any reports concerning attempts to incorporate in a transient manner (prodrug) the functional elements of penetration enhancers (such as sulfoxide or amide groups) directly into the steroid that is to be delivered. Indeed, this approach has already been shown to be effective in enhancing the delivery of non-steroidal anti-inflammatory agents through biological membranes. Thus, Loftsson and Bodor (1981) have shown that the methylsulfinylmethyl (a sulfoxide) prodrug of aspirin approximately doubles the topical delivery of aspirin through hairless mouse skin. Similarly, Sloan (1980) has shown that the use of the N,N-diethylhydroxylamine (an amine) derivative of indomethacin doubles the delivery of indomethacin through hairless mouse skin from a plastibase topical formulation [1].

However, it should be pointed out that a general mechanism whereby penetration enhancers act is not known with certainty. Apparently though, this action is due to a change in the skin that only an excess (compared to drug being delivered) of the penetration enhancer can cause (Allenby et al., 1969b). Therefore, if the incorporation of the functional elements of penetration enhancers into prodrugs succeeds in improving the delivery of drugs through biological membranes, it succeeds for different reasons than that the penetration enhancers themselves succeed. In this case, it would probably result because of changes in the physical properties of the drug, such as improved biphasic solubility caused by the derivatization.

In addition to changing the physical properties of the drug and hence its diffusion rate through skin, the prodrug will also affect the activity and toxicity of the drug by changing its distribution and its rate of presentation to sites where its activities are manifested. Therefore, in order to determine if the transient incorporation of biphasic solubilizing groups into hydrocortisone would improve the delivery of hydrocortisone through skin and, concomitantly, its topical activity, a number of esters of hydrocortisone containing sulfoxide and amide groups have been prepared and their diffusional properties and biological activities have been compared to that of hydrocortisone. The preliminary results of those studies involving primarily derivatives containing an amide group are reported here.

[1] Sloan, K., Selk, S., Haslam, J. and Caldwell, L., unpublished results.

Materials and Methods

The hairless mice that were used were SKH-hr-1 mice from Temple University Skin and Cancer Hospital. The diffusion cells were obtained from Kercso Engineering Consultants, Palo Alto, CA. TLC were run on Brinkman Polygram Sil G/UV 254. MP (uncorrected) were taken with a Thomas-Hoover Capillary Apparatus. NMR spectra were recorded on a Varian T-60, IR spectra on a Beckman Acculab 4 spectrophotometer and the optical rotations were obtained using a Perkin Elmer 141 polarimeter. Microanalyses were performed by Midwest Microlab, Indianapolis, IN. The hydrocortisone, hydrocortisone-21-acetate, prednisone, dexamethasone and triamcinolone acetonide were obtained from Sigma. The remaining reagents were obtained from Aldrich while the bulk solvents were obtained from Mallinckrodt. Trimethylorthovalerate was obtained from Eastman Chemicals. The triethylorthobutyrate was prepared according to the iminoester method (McElvain and Stevens, 1947) and the NMR spectrum of the product was consistent with the assigned structure. The hydrocortisone-17-propionate, -17-butyrate and -17-valerate were prepared according to the general method of Gardi et al. (1963) for the preparation of the steroidal orthoester and according to the general method of Salce et al. (1970) for the conversion of the steroid orthoester to the steroid 17-ester.

4-Pregnene-11β,21-dihydroxy-17α-propionyloxy-3,20-dione (III): m.p. 181–184°C from methanol–water (3 : 1) in 32% yield; $[\alpha]^{24}D + 63.5°C$, (C = 0.5, ethanol); ^1H NMR ($CDCl_3$)δ5.67 (s, 1, O=C–C<u>H</u>=C), 4.27 (s, 2, O=C–C<u>H</u>$_2$OH), 1.47 (s, 3, C<u>H</u>$_3$) and 0.95 (s, 3, C<u>H</u>$_3$). Anal: Calcd. for $C_{24}H_{33}O_6$: C, 69.04; H 7.97. Found: C, 68.80; H, 8.30.

4-Pregnene-11β,21-dihydroxy-17α-butyryloxy-3,20-dione (IV): m.p. 203–206°C from methanol–water (4 : 1) in 58% yield, lit. m.p. 208–210°C (Vitali et al., 1966); $[\alpha]^{25}D + 114.5°C$, (C=0.5, dioxane); TLC (Silica gel, ether) R_f 0.12; ^1H NMR ($CDCl_3$) δ5.71 (s, 1, O=C–C<u>H</u>=C), 4.33 (d, 2, J = 5 Hz, O=C–C<u>H</u>$_2$OH), 1.47 (s, 3, C<u>H</u>$_3$) and 0.97 (s, 3 C<u>H</u>$_3$–C). Anal: Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.48; H, 8.49.

4-Pregnene-11β,21-dihydroxy-17α-valeryloxy-3,20-dione (V): m.p. 158–175°C from methanol in 51% yield; $[\alpha]^{24}D + 61.5°C$, (C = 0.46, ethanol); ^1H NMR ($CDCl_3$) δ5.71 (s, 1, O=C–C<u>H</u>–C) and 4.29 (d, 2, J = 5 Hz, O=CC<u>H</u>$_2$OH). Anal: Calcd. for $C_{26}H_{36}O_6$: C, 70.08; H, 8.60. Found: C, 70.00; H, 8.55.

The preparation of amidocarboxylic acids

The various amidocarboxylic acids were prepared according to the method of Pauling (Pressman et al., 1949). Thus, the following amidocarboxylic acids were prepared by mixing equal portions of the required secondary amine with ice-cold dichloromethane solutions of succinic or diglycolic anhydride. The solutions were then concentrated and the oils distilled or the solids recrystallized to give the following amidocarboxylic acids.

N,N-Dimethylsuccinamic acid (VI): m.p. 86–89°C from tetrahydrofuran, lit. m.p. 81.6–82.6°C (Pressman et al., 1948), 47% yield. Anal: Calcd. for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N 9.65. Found: C, 50.00; H, 7.83; N, 9.74.

N,N-Diethylsuccinamic acid (VII): m.p. 79–82°C from dichloromethane–heptane (1 : 4.5), lit. m.p. 82.1–84.1°C (Pressman et al., 1949), 85% yield. Anal: Calcd. for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.62; H, 8.83; N, 8.03.

N,N-Dipropylsuccinamic acid (VIII): m.p. 35–37°C from dichloromethane. Anal: Calcd. for $C_{10}H_{19}NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.42; H, 9.59; N, 6.97.

N,N-Dibutylsuccinamic acid (IX): an oil in 56% yield. Anal: Calcd. for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11. Found: C, 63.05; H, 10.40; N, 6.01.

N,N-Dihexylsuccinamic acid (X): an oil in 81% yield. Anal: Calcd. for $C_{16}H_{31}NO_3$: C, 67.32; H, 10.95; N, 4.91. Found: C, 67.00; H, 10.89; N, 5.18.

N,N-Diethyl-3-oxaglutaramic acid (XI): From diglycolic anhydride as an oil in 79% yield.

N,N-Tetramethylenesuccinamic acid (XII): m.p. 103–108°C from dichloromethane–ether (75 : 200) in 87% yield. Anal: Calcd. for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18. Found: C, 55.88; H, 7.52; N, 8.05.

N,N-3-Methylazapentamethylenesuccinamic acid (XIII): m.p. 122–127°C from tetrahydrofuran–ether. Anal: Calcd. for $C_9H_{16}N_2O_3$: C, 53.98; H, 8.05; N, 13.99. Found: C, 53.72; H, 8.08; N, 13.78.

N,N-3-Oxapentamethylenesuccinamic acid (XIV): m.p. 73–78°C from tetrahydrofuran–ether. Anal: Calcd. for $C_8H_{13}NO_4$: C, 51.32; H, 7.00; N, 7.48. Found: C, 51.54; H, 7.12; N, 7.35.

The preparation of amidocarboxylic esters of glucocorticoid steroids

The steroid esters were prepared by dissolving the steroid in pyridine (10 ml/0.01 mol) and allowing that solution to react with equivalent molar amounts of the amidocarboxylic acid and dicyclohexylcarbodiimide. After the reaction had been stirred at room temperature for 10 min, it was diluted with dichloromethane (20 ml/0.01 mol) and the suspension that was obtained was stirred at room temperature overnight. The suspension was filtered and the filtrate was concentrated in vacuo to remove the pyridine. The concentrate was then either recrystallized or chromatographed on silica gel (Mallinckrodt CC-7) then recrystallized to give the following esters.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-dimethylsuccinamoyloxy)-3,20-dione (XV): m.p. 228–233°C from tetrahydrofuran in 35% yield; TLC (silica gel, ether–methanol, 10 : 3) R_f 0.5; $[\alpha]^{25}D$ + 127°C, (C = 0.5, dioxane). Anal: Calcd. for $C_{27}H_{39}NO_7$: C, 66.23; H, 8.03; N, 2.86. Found: C, 66.41; H, 8.35; N, 2.68.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-diethylsuccinamoyloxy)-3,20-dione (XVI): m.p. 100–105°C from tetrahydrofuran–ether (50 : 225) in 64% yield; TLC (silica gel, acetone) R_f 0.37; $[\alpha]^{23.5}D$ + 139°C, (C = 0.55, ethanol). Anal: Calcd. for $C_{29}H_{43}NO_7$: C, 67.38; H, 8.56; N, 2.70. Found: C, 67.55; H, 8.75; N, 2.65.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-dipropylsuccinamoyloxy)-3,20-dione (XVII): m.p. 157–162°C from tetrahydrofuran in 37% yield; TLC (silic gel, ether–methanol, 10 : 1) R_f 0.56; $[\alpha]^{25}D$ + 127°C, (C = 0.5, dioxane). Anal: Calcd. for $C_{31}H_{47}NO_7$: C, 68.23; H, 8.68; N, 2.57. Found: C, 68.48; H, 9.02; N, 2.23.

4-Pregnene-11β-17α-dihydroxy-21-(N,N-dibutylsuccinamoyloxy)-3,20-dione

(XVIII): m.p. 173–174°C, chromatographed then crystallized from tetrahydrofuran–ether in 59% yield; $[\alpha]^{26}D + 120°C$, (C = 0.5, ethanol). Anal: Calcd. for $C_{33}H_{51}NO_7$: C, 69.08; H, 8.96; N, 2.44. Found: C, 69.09; H, 8.93; N, 2.47.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-dihexylsuccinamoyloxy)-3,20-dione (XIX): m.p. 148–149°C, chromatographed then crystallized from tetrahydrofuran–ether in 44% yield; $[\alpha]^{26}D + 107°C$, (C = 0.5, ethanol). Anal: Calcd. for $C_{37}H_{59}NO_7$: C, 70.55; H, 9.44; N, 2.22. Found: C, 70.26; H, 9.31; N, 2.40.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-diethyl-3'-oxaglutaramoyloxy)-3,20-dione (XX): m.p. 95–97°C from tetrahydrofuran–ether, 20 : 80 in 29% yield; TLC (silica gel, ether–methanol, 10 : 1) R_f 0.36; $[\alpha]^{23}D + 117°C$, (C = 0.5, dioxane). Anal: Calcd. for $C_{29}H_{43}NO_8 \cdot C_2H_5OC_2H_5$: C, 65.21; H, 8.79; N, 2.30. Found: C, 65.15; H, 8.60; N, 2.06.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-tetramethylenesuccinamoyloxy)-3,20-dione (XXI): m.p. 152–155°C from tetrahydrofuran; TLC (silica gel, ether-methanol, 10 : 3) R_f 0.53; $[\alpha]^{25}D + 116°C$, (C = 0.47, dioxane). Anal: Calcd. for $C_{29}H_{41}NO_7 \cdot 0.5\ H_2O$: C, 64.18; H, 8.17; N, 2.58. Found: C, 64.24; H, 7.94; N, 2.21.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-3'-methylazapentamethylenesuccinamoyloxy)-3,20-dione (XXII): m.p. 159–162°C from dichloromethane in 51% yield; TLC (silica gel, methanol) R_f 0.27; $[\alpha]^{24}D + 122°C$, (C = 0.5, dioxane). Anal: Calcd. for $C_{30}H_{44}N_2O_7$: C, 66.16; H, 8.14; N, 5.14. Found: C, 66.22; H, 8.21; N, 5.04.

4-Pregnene-11β,17α-dihydroxy-21-(N,N-3'-oxapentamethylenesuccinamoyloxy)-3,20-dione (XXIII): m.p. 223–225°C from methanol; TLC (silica gel, ether–methanol, 10 : 1) R_f 0.29; $[\alpha]^{25}D + 131°C$, (C = 0.5, dioxane). Anal: Calcd. for $C_{29}H_{41}NO_8$: C, 65.51; H, 7.77; N, 2.64. Found: C, 65.46; H, 7.99; N, 2.51.

4-Pregnene-11β-hydroxy-21-(N,N-diethylsuccinamoyloxy)-17α-propionyloxy-3,20-dione (XXIV): m.p. 96–103°C from ether–dichloromethane–cyclohexane in 48% yield after initial chromatography; $[\alpha]^{25}D + 73°C$, (C = 0.5, ethanol). Anal: Calcd. for $C_{32}H_{47}NO_8$: C, 66.99; H, 8.26; N, 2.44. Found: C, 66.85; H, 8.27; N, 2.14.

4-Pregnene-11β-hydroxy-21-(N,N-diethylsuccinamoyloxy)-17α-butyryloxy-3,20-dione (XXV): m.p. 81–83°C from dichloromethane–heptane, 16 : 6 in 44% yield after initial chromatography; $[\alpha]^{26.5}D + 67°C$, (C = 0.5, dioxane); TLC (silica gel, ether) developed twice R_f 0.25. Anal: Calcd. for $C_{33}H_{49}NO_8 \cdot 0.5\ H_2O$: C, 66.41; H, 8.44; N, 2.34. Found: C, 66.76; H, 8.56; N, 2.00.

4-Pregnene-11β-hydroxy-21-(N,N-diethylsuccinamoyloxy)-17α-valeryloxy-3,20-dione (XXVI): m.p. 126–128°C from dichloromethane–cyclohexane in 69% yield after initial chromatography; $[\alpha]^{26}D + 68°C$, (C = 0.5, ethanol). Anal: Calcd. for $C_{34}H_{51}NO_8$: C, 67.86; H, 8.54; N, 2.33. Found: C, 67.54; H, 8.68; N, 2.00.

1,4-Pregnadiene-17α-hydroxy-21-(N,N-diethylsuccinamoyloxy)-3,11,20-trione (XXVII): m.p. 204–206°C from tetrahydrofuran in 47% yield; $[\alpha]^{24}D + 144.5°C$, (C = 0.55, dioxane). Anal: Calcd. for $C_{29}H_{39}NO_7$: C, 67.81; H, 7.65; N, 2.73. Found: C, 68.15; H, 7.80; N, 2.40.

1,4-Pregnadiene-11β,17α-dihydroxy-9α-fluoro-16α-methyl-21-(N,N-diethylsuccinamoyloxy)-3,20-dione (XXVIII): m.p. 202–204°C from tetrahydrofuran in 42% yield; $[\alpha]^{24}D + 68°C$, C = 0.5, dioxane). Anal: Calcd. for $C_{30}H_{42}NO_7F$: C, 65.79; H, 7.73; N, 2.56. Found: C, 66.15; H, 7.80; N, 2.40.

The preparation of 4-pregnene-11β,21-dihydroxy-17α-(N,N-diethylsuccinamoyloxy)-3,20-dione (XXIX)

XXIX was prepared according to the general procedure of Phillips et al. (1974). To a suspension of 0.456 g (0.0024 mol) of cuprous iodide in 20 ml of dry tetrahydrofuran at 0°C was added 2.66 ml of 1.6 M methyl lithium (0.0043 mol) in ether. The reaction mixture was stirred at 0°C for 15 min then the temperature was reduced to −30°C and 4-pregnene-11β,17α-dihydroxy-21-(N,N-diethylsuccinamoyloxy)-3,20-dione (0.50 g, 0.00096 mol) was added. The reaction was stirred at −25°C for 45 min then 1.53 g of N,N,N′,N′-tetramethylethylenediamine tetrachlorocuprate was added and the reaction mixture was allowed to warm to room temperature and stirred overnight at room temperature. The reaction was then poured into 150 ml of 3 M NH_4Cl and stirred for 1 h. The oily precipitate was extracted with ethyl acetate and chromatographed on silicAR CC-7 using methanol–ether 10 : 90 as the eluent to give crystals (0.12 g, m.p. 160–170°C, from methanol) of the desired product: IR (KBr) 3460 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ5.66 (m, 1, O=C–C<u>H</u>=C), 4.6–4.3 (m, 1, C<u>H</u>–OH), 4.27 (s, 2, O=CC<u>H</u>$_2$–OH), 3.5–3.1 (m, 4, NC<u>H</u>$_2$CH$_3$), 2.60 (sharp m, 4, O=CC<u>H</u>$_2$C<u>H</u>$_2$C=O), 1.61 (s, 3, C<u>H</u>$_3$–C), 0.95 (s, 3, C<u>H</u>$_3$–C), 1.17 (t, J = 6Hz, 6, N–CH$_2$C<u>H</u>$_3$), 3.0–0.8 (m, 19, C<u>H</u>$_2$, C<u>H</u> and O<u>H</u>). Anal: Calcd. for $C_{29}H_{43}NO_7 \cdot H_2O$: C, 65.02; H, 8.46; N, 2.61. Found: C, 64.96; H, 8.30; N, 2.58.

The preparation of 4-pregnene-11β,17α-dihydroxy-21-(pyrrolid-2′-one-5′-carboxylate)-3,20-dione (XXX)

A mixture of 21.6 g (0.06 mol) of hydrocortisone, 7.8 g (0.06 mol) of 2-pyrrolidone-5-carboxylic acid and 12.6 g (0.06 mol) of dicyclohexylcarbodiimide was dissolved in 40 ml of pyridine. A vigorous reaction resulted which caused the solution to solidify. The solid was suspended in 375 ml of dichloromethane overnight, then filtered. The filtrate was concentrated in vacuo to a foam which was redissolved in 50 ml of dichloromethane and filtered. The dichloromethane solution was diluted with 600 ml of ether. The gummy solid suspension that resulted gradually became a fine white powder after the suspension was stirred for 2 h. The suspension was then filtered and dried in vacuo at 60°C for 10 h to give 22.1 g (m.p. 140–142°C, 77% yield) of the desired compound: TLC (silica gel, acetone) R_f 0.46; IR (KBr) 3400 cm^{-1} (m) (broad OH and NH) and 1650 cm^{-1} (s) (broad C=O); ^1H NMR ($CDCl_3$) δ6.67 (s, 1, N–<u>H</u>), 5.63 (s, 1, O=C–C<u>H</u>=C), 5.03 (broad s, 2, O=C–C<u>H</u>$_2$O), 4.6–4.3 (m, 2, C<u>H</u>–OH and O$_2$C–C<u>H</u>–N), 1.43 (s, 3, C<u>H</u>$_3$–C), 0.90 (s, 3, C<u>H</u>$_3$–C) and 3.2–0.7 (m, 21, C<u>H</u>$_2$, C<u>H</u>, O<u>H</u>); $[\alpha]^{27}D$ + 131°C, (C = 1.0, methanol). Anal: Calcd. for $C_{26}H_{35}NO_7$: C, 65.94; H, 7.45; N, 2.96. Found: C, 65.86; H, 7.54; N, 3.27.

The preparation of 4-pregnene-11β,17α-dihydroxy-21-(2′,2′-dimethyl-3′-formyl-1′,3′-thiazolidyl-4′-carboxylate)-3,20-dione (XXXI)

To 1.8 g (0.005 mol) of hydrocortisone dissolved in 5 ml of pyridine was added 1.05 g (0.005 mol) of dicyclohexylcarbodiimide and 0.95 g (0.005 mol) of 4-carboxy-3-formyl-2,2-dimethylthiazolidine (Sheehan and Yang, 1958). Immediately, a waxy solid precipitated. Dichloromethane (20 ml) was added and the suspension that

resulted was stirred overnight at room temperature. The suspension was filtered and the filtrate was concentrated in vacuo to give a hard foam. The foam was dissolved in tetrahydrofuran (10 ml) and the suspension that resulted was filtered. The tetrahydrofuran filtrate was diluted to 75 ml with ether. After 16 h, the solution was decanted from an oil that formed and 20 ml of cyclohexane was added. The crystals that resulted were filtered and dried in vacuo to give 1.40 g (m.p. 175–180°C, 52% yield) of the desired ester: TLC (Silica gel, ether–CH_3OH, 10:2) R_f 0.50; IR (KBr) 3460–3420 cm^{-1} (s) (OH) and 1740, 1710, 1645 and 1630 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ8.31 (s, 1, NC<u>H</u>=O), 5.67 (s, 1, O=C–C<u>H</u>=C), 5.05 (s, 2, O=C–C<u>H</u>$_2$–O$_2$), 5.25–4.9 (m, 1, O$_2$C–C<u>H</u>–N), 4.6–4.3 (m, 1, C<u>H</u>–OH), 3.6–3.4 (m, 2, C<u>H</u>$_2$–S), 1.83 (s, 6, (C<u>H</u>$_3$)$_2$–C), 1.48 (s, 3, C<u>H</u>$_3$–C), 0.91 (s, 3, C<u>H</u>$_3$–C) and 3.6–0.7 (m, 19, O<u>H</u>, C<u>H</u>$_2$ and C<u>H</u>); $[\alpha]^{24.5}D$ + 46.6°C, (C = 0.5, ethanol). Anal: Calcd. for $C_{28}H_{39}NO_7S$: C, 63.02; H, 7.37; N, 2.63. Found: C, 63.33; H, 7.50; N, 2.40.

The preparation of 4-pregnene-11β,17α-dihydroxy-21-(2',2'-dimethyl-3'-formyl-1'-oxo-1',3'-thiazolidyl-4'-carboxylate)-3,20-dione (XXXII)

4-Pregnene-11β,17α-dihydroxy-21-(2',2'-dimethyl-1',3'-thiazolidyl-4'-carboxylate)-3,20-dione (3 g, 5.62 mmol) and *m*-chloroperoxybenzoic acid (0.97 g, 5.62 mmol) were dissolved in 160 ml of chloroform and stirred overnight at room temperature. The reaction was extracted with 100 ml of 0.5 N NaOH then with 100 ml of water. The organic layer was separated and dried with Na_2SO_4 and filtered. The filtrate was concentrated and absorbed onto 20 g of SilicAR CC-7 and chromatographed on 80 g of CC-7 using 10% tetrahydrofuran in heptane to 100% tetrahydrofuran as eluent. The fraction containing the product was concentrated to give 3.07 g (99% yield) of slightly yellow powder which was crystallized overnight from 10 ml of hot tetrahydrofuran to give 1.39 g (45% yield, m.p. 243–245°C) of white needles which were the desired product: ^1H NMR ($CDCl_3$ + DMSO-d_6) δ8.37 (s, 1, N–C<u>H</u>O), 5.60 (s, 1, O=C–C<u>H</u>=C), 5.42 (t, 1, N–C<u>H</u>–C=O), 5.08 (s, 2, O=C–C<u>H</u>$_2$O–), 1.84 (s, 3, <u>H</u>$_3$C–C(N)S–), 1.52 (s, 3, <u>H</u>$_3$C–C(N)S–), 1.43 (s, 3, C<u>H</u>$_3$), 0.83 (s, 3 ,C<u>H</u>$_3$); IR (KBr) 1764, 1724 cm^{-1} (s) (O–C=O), 1658 cm^{-1} (s) (O=C–C=C), 1058 cm^{-1} (m) (S → O); $[\alpha]^{24}D$ + 29°C, (C = 0.55, ethanol). Anal: Calcd. for $C_{28}H_{41}NO_9S$: C, 59.23; H, 7.28; N, 2.46. Found: C, 59.55; H, 7.26; N, 2.10.

Biological studies

The biological studies were performed according to the general procedures previously described in detail (Bodor et al., 1982). Thus, the systemic effect of the steroids on the thymus weight of weanling rats was determined according to the method of Tonelli et al. (1965); the blanching effect of the steroids on human volunteers was obtained using the general procedure of McKenzie and Stoughton (1962); the local effect of the steroids was determined using a modification of the method of Smith et al. (1976); the ability of the steroids to reduce swelling in a mouse ear model caused by croton oil was measured using the general method of Tonelli et al. (1965); and finally the diffusion cell experiments were carried out according to the previously described procedure (Bodor et al., 1982) using hairless mice and Kersco diffusion cells.

Results and Discussion

The steroid amides were prepared by dissolving the steroid in pyridine and then adding the amidocarboxylic acid and dicyclohexylcarbodiimide to the solution. After about 10 min, the reactions were diluted with dichloromethane; otherwise, significant quantities (up to 40% in some cases) of the corresponding N-acyldicyclohexylureas were isolated. This unusual stepwise addition of solvents was found to be the best procedure because of the properties of the solvents. For instance, although pyridine as a solvent has been reported to catalyze the rearrangement of the O-acylisourea intermediate (Hegarty et al., 1977) to non-productive N-acylureas (vide ante) in the acylation reactions of carbodiimides (Kurzer and Douraghi-Zadeh, 1967), smaller amounts of pyridine have been reported to enhance the formation of esters from the same reaction intermediate, i.e. O-acylisourea [one equivalent in tetrahydrofuran or dichloroethane (Buzas et al., 1963); 0.25 equivalent in acetonitrile (DeTar and Silverstein, 1966)]. In addition, pyridine was the best solvent available to maintain the necessary homogeneous reaction conditions in the reactions. On the other hand, dichloromethane was a poor solvent for the steroids and by itself was a poor solvent in which to run the reactions (less than half the yields were obtained), but as a diluent for pyridine, it did not itself enhance any O- to N-acyl rearrangement. Thus, the combination of the two solvents gave by far the best results.

Other reaction schemes were also tried. The sequence used for testosterone (Kupchan et al., 1965) could not be used here because it was not selective enough to acylate only the 21-hydroxy group in the more complicated glucocorticoid series. The acid chlorides of the amidocarboxylic acids were also tried but they were found not to lead to the desired product probably because of competing intramolecular reactions (Sloan and Bodor, 1982).

Most of the amidocarboxylic esters that were prepared were based on succinamic acid; however, there were two exceptions. 2-Pyrrolidone-5-carboxylic acid is a known humectant which has been shown to increase the water-holding capacity of the skin (Middleton and Roberts, 1978). Consequently, numerous esters of 2-pyrrolidone-5-carboxylic acid have been made (Eberhardt et al., 1974) for use in dermatological preparations. Esters based on 2-pyrrolidone-5-carboxylic acid contain a secondary amide rather than a tertiary amide and it was of interest to see what effect that variation might have on activity. However, although it was possible to prepare the 2-pyrrolidone-5-carboxylic acid ester of hydrocortisone (XXX), the ester was extremely hygroscopic and decomposed in the solid state even on storage in a desiccator.

The other unusual amidoester prepared was the N-formyl thiazolidine ester (XXXI). The intent here was to incorporate, in addition to a tertiary amide group, a sulfoxide group as well — a functional group present in dimethylsulfoxide which is known to facilitate the absorption of steroids through the skin (Maibach and Feldmann, 1967). However, when the sulfide was converted to the sulfoxide XXXII, there was a considerable increase in the melting point of the steroid derivative and a concomitant decrease in the solubility of the derivative in most organic solvents. Subsequently, the sulfoxide was screened in the blanching test and found to be inactive.

TABLE 1

SPECTRAL PROPERTIES OF AMIDOCARBOXYLIC ACIDS

	NMR (δ) [a]		IR (cm^{-1}) [b]	
	O=C-N($C\underline{H}_2$R)	$C\underline{H}_2$-C=O-X [c]	O=C-O	O=C-N
VI	3.05, 2.96 (2×3s)	2.67 (4s)		
VII	3.35 (4m)	2.67 (4s)		
VIII	3.28 (4t)	2.65 (4s)	1725	1630
IX	3.32 (4m)	2.69 (4s)	1730	1610
X	3.3 (4m)	2.65 (4s)	1725	1620
XI	3.3 (4m)	4.43 ($HO_2CC\underline{H}_2$) 4.20 ($R_2NCOC\underline{H}_2$)	1730	1620
XII	3.5 (4m)	2.63 (4s)		
XIII [d]	3.87 (4s)	2.57 (4m)	1630 [e]	1600
XIV [d]	3.67 (8s)	2.68 (4s)	1730	1605

[a] Run in $CDCl_3$ unless otherwise stated.
[b] KBr or neat.
[c] Where X can be oxygen or nitrogen.
[d] NMR in D_2O.
[e] 1630 cm^{-1} band due to CO_2^-.

The spectral properties of amidocarboxylic acids and their respective steroid esters are recorded in Tables 1 and 2. The spectral data are consistent with the assigned structures.

The steroid amidocarboxylic acid esters were all screened either in the blanching

TABLE 2

SPECTRAL PROPERTIES OF AMIDE ESTERS OF STEROIDS

	NMR (δ) [a]					
	$C\underline{H}_3$(s)	$C\underline{H}_3$(s)	O=C-$C\underline{H}$=C (s)	21-$C\underline{H}_2$	O=C-N($C\underline{H}_2R)_2$	-$C\underline{H}_2$-C=O-X
XV	1.45	0.97	5.7	4.97 (2s)	3.2, 3.1 (2×3s)	2.7 (4m)
XVI	1.45	0.97	5.65	4.93 (2s)	3.33 (4q, J = 7Hz)	2.7 (4m)
XVII	1.46	0.96	5.64	4.93 (2m)	3.24 (4t, J = 7HZ)	2.7 (4s)
XVIII			5.69	5.02 (2s)	3.30 (4m)	2.73 (4s)
XIX			5.66	4.96 (2s)	3.27 (4m)	2.71 (4s)
XX	1.44	0.93	5.67	5.07 (2s)	3.34 (4m)	4.35, 4.3 (2×2s)
XXI	1.43	0.93	5.63	4.93 (2s)	3.35 (4m)	2.63 (4m)
XXII [b]	1.37	0.78	5.53	4.91 (2m)	3.41 (8m)	
XXIII [b]	1.38	0.79	5.55	4.93 (2m)	3.50 (8m)	2.62 (4s)
XXIV			5.73	4.68 (2m)	3.34 (4m)	
XXV	1.37		5.65	4.65 (2m)	3.30 (4m)	2.60 (4s)
XXVI			5.70	4.70 (2m)	3.34 (4m)	2.70 (4s)
XXVII	1.47	0.67	6.07	4.90 (2m)		
XXVIII	1.57	1.05	6.10	4.93 (2s)		

[a] All spectra run in $CDCl_3$ unless otherwise noted.
[b] DMSO-d_6

TABLE 3

CROTON OIL IRRITATION TEST [a]

Compound	ED_{50}(M) [b]		
	Exp. 1	Exp. 2	Exp. 3
XV		0.0143	
XVII		0.0013	
XX		0.0055	
XXI		0.379	
XXII		0.013	
XXIII		0.0176	
XXIV	0.0011		
XXV		0.0008	
XXVI	0.0019		
XXXI		0.0018	
Hydrocortisone-17-butyrate	0.0012	0.0011	0.00086
Hydrocortisone			0.0084
Hydrocortisone-21-acetate			0.016

[a] 10 mice used in each experiment for each steroid. 50 μl of an acetone–2% croton oil solution of steroid applied.

[b] Based on linear regression analyses of data obtained at 3×10^{-5}, 3×10^{-4}, 3×10^{-3}, and 3×10^{-2} M using hydrocortisone-17-butyrate as the internal standard in each experiment. Data generated at the laboratories of Kanebo Pharmaceuticals, Japan.

test or the croton oil irritation test in mice. In the mouse ear assay (Table 3), it was found that of the simple esters, the dipropylsuccinamate was the most active; it was almost as active as the hydrocortisone-17α-butyrate and about 8 times as active as hydrocortisone, taking into account normalization across the two experiments.

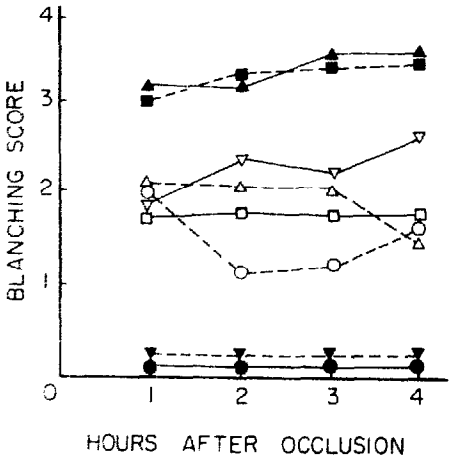


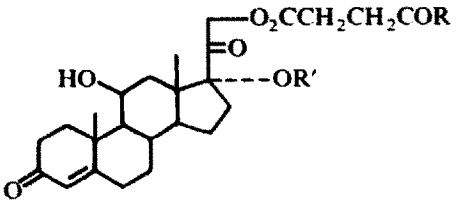
Fig. 1. Blanching in human volunteers after topical application of 100 μl of acetone–isopropyl myristate (90:10) solutions, 0.03 M in the steroids. The scoring criteria of Barry and Woodford (1974) was used. The blanching data are the average of the blanching scores for 4 applications onto each of 4 volunteers (average, S.D. ± 0.25); ▲, hydrocortisone 17-valerate; ■, hydrocortisone 17-butyrate; △, hydrocortisone 21-acetate; □, hydrocortisone; ▼, XVIII; ●, XIX; ▽, XVI; ○, XVII.

However, in the blanching study (Fig. 1) the diethylsuccinamate was found to be the most active. It was nearly twice as active as the dipropyl derivative but not significantly more active than hydrocortisone 21-acetate. Also shown in Fig. 1 are the results of the blanching test on the dibutyl and dihexylsuccinamate esters; they were completely inactive. Not shown are the results for the 17α-diethylsuccinamate ester; it was found to be no more active than the 21-ester in the blanching test.

Of the mixed 21,17α-diesters represented by XXIV, XXV and XXVI the 17α-valerate-21-diethylsuccinamate (XXVI) was only about 60% as active as the 17α-butyrate, while the 17α-butyrate-21-diethylsuccinamate (XXV) was 30% more active and the 17α-propionate-21-diethylsuccinamate (XXIV) was about as active as hydrocortisone-17α-butyrate in the mouse ear assay. Although the results for the blanching tests with the mixed esters have not been presented, the following qualitative results were obtained consistently. In the blanching test XXVI was more

TABLE 4

THYMUS INVOLUTION IN RATS AFTER TOPICAL APPLICATION OF STEROIDS [a]



Steroid	R	R'	mg of thymus/100 g of rat ± S.D. (% reduction in weight of thymus) [b]		
			Exp. 1	Exp. 2	Exp. 3
XV	$N(CH_3)_2$	H	183 ± 42 (30)		
XVI	$N(C_2H_5)_2$	H	160 ± 25 (39)		
XVII	$N(C_3H_7)_2$	H	141 ± 44 (46)		
XVIII	$N(C_4H_9)_2$	H	176 ± 38 (33)		
XIX	$N(C_6H_{13})_2$	H	243 ± 44 (7)		
XXIV	$N(C_2H_5)_2$	COC_2H_5		139 ± 31 (32)	
XXV	$N(C_2H_5)_2$	COC_3H_7			166 ± 12 (38)
XXVI	$N(C_2H_5)_2$	COC_4H_9		177 ± 28 (16) [d]	
I, Hydrocortisone			168 ± 30 (36)	140 ± 42 (33)	223 ± 10 (17)
II, Hydrocortisone-21-acetate [d]			167 ± 18 (36)		
III, Hydrocortisone-17-propionate				120 ± 26 (43)	
IV, Hydrocortisone-17-butyrate				120 ± 19 (43)	187 ± 7 (30)
V, Hydrocortisone-17-valerate			206 ± 20 (21) [c]	159 ± 29 (24)	
Triamcinolone acetonide					49 ± 4 (82)
Control (vehicle)			262 ± 38	210 ± 44	268 ± 14

[a] All compounds were administered in a total of 50 μl as a 0.03 M acetone/IPM (90:10) solution, except where specified, to 10 rats each, 100 rats at a time. (IPM = isopropyl myristate.)

[b] Compared to control.

[c] Tetrahydrofuran/IPM (90:10) used as a vehicle to apply steroid.

[d] $P < 0.1$ compared to hydrocortisone-17α-valerate.

[e] $P < 0.01$ compared to hydrocortisone.

active than the 21-diethylsuccinamate XVI but not significantly so, while it was impossible to separate the activity of the hydrocortisone-17α-esters studied (17α-butyrate, 17α-propionate, 17α-valerate) from the blanching activity of the 17α-butyrate or the 17α-propionate-21-diethylsuccinamates under the conditions used.

The results from the determination of the systemic toxicity of the steroid esters are recorded in Table 4. Of the simple amidocarboxylic acid esters only the dihexylsuccinamate was significantly less toxic than hydrocortisone or hydrocortisone-21-acetate, but it was totally inactive in the blanching test. The mixed esters gave more interesting results. First, it is interesting that the 17α-valerate is not only less toxic systemically than hydrocortisone or the other 17α-esters but that it is also significantly less toxic locally than the other 17α-esters (Bodor et al., 1982). Second, it is interesting that the 17α-valerate-21-diethylsuccinamate (XXVI) was significantly less toxic systemically than the relatively non-toxic 17α-valerate itself. The other two mixed esters were either comparable in systemic toxicity to hydrocortisone (XXIV) or more toxic (XXV). Thus, although XXVI was not as active as the other 17α-ester-21-diethylsuccinamate esters in either the croton oil assay or the blanching studies, it was significantly less toxic systemically than the 17α-esters or hydrocortisone and it was more active than hydrocortisone or its 21-acetate.

In order to see what effect the incorporation of the amidocarboxylate group had on the diffusion and on the local toxicity of hydrocortisone, a representative simple ester was chosen for further study. Among the simple esters the diethylsuccinamate had been found to be the most active ester in the blanching test so it was the one chosen for further study. In Table 5 the effect of XVI is compared with the effect of hydrocortisone, etc., on the double-fold skin thickness of hairless mice. XVI is significantly less toxic in this test than either hydrocortisone or hydrocortisone-21-

TABLE 5

EFFECTS OF STEROIDAL ANTI-INFLAMMATORY AGENTS ON THE DOUBLE-FOLD SKIN THICKNESS OF HAIRLESS MICE

Steroid [a]	Skin thickness (in. × 10^4 ± S.E.)	
	Exp. 1 [b]	Exp. 2 [c]
Control (propylene glycol)	120 ± 2	125 ± 3
Triamcinolone acetonide	65 ± 4 [d]	
IV. Hydrocortisone-17-Butyrate	73 ± 2	
II. Hydrocortisone-21-Acetate	75 ± 2	
XVI	88 ± 2	94 ± 3 [e]
I. Hydrocortisone		79 ± 2

[a] 50 μl of a 0.03 M propylene glycol solution once each day for 30 days.

[b] n = 9.

[c] n = 10.

[d] 6 animals died during the course of the treatment (n = 3).

[e] Significantly greater than hydrocortisone or hydrocortisone-21-acetate, $P < 0.05$.

TABLE 6

STEROID DIFFUSION THROUGH FRESH HAIRLESS MOUSE SKIN

Steroid [a]	Mol% hydrocortisone [b] diffused ± S.D. [c,d] (hour after application)			
I	2.1 ± 0.8 (4)	4.1 ± 1.3 (9)	7.5 ± 1.8 (18)	8.4 ± 3.0 (24)
II	1.7 ± 0.6 (4)	3.5 ± 1.3 (9)	6.2 ± 1.3 (18)	14.1 ± 5.9 (24)
XVI	1.8 ± 0.5 (3.5)	6.1 ± 3.7 (10.5)	18.0 ± 6.7 (19)	23.1 ± 8.7 (24)

[a] 50 μl of a 0.03 M acetone/isopropylmyristate (90 : 10) solution of the steroid was applied to the skin.
[b] Only hydrocortisone was found on the receptor side of the membrane under conditions where hydrocortisone-21-acetate or the succinamate ester could have been detected.
[c] n = 3.
[d] The slope ± S.D. in mol%/h for the rate of diffusion for each compound is 0.32 ± 0.26 (I), 0.57 ± 0.11 (II), and 1.09 ± 0.08 (XVI).

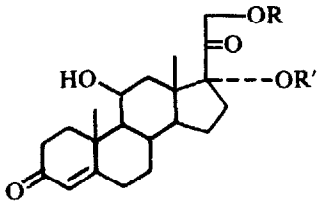
acetate, either of which are considered safe enough for sale over the counter at the same concentrations (about 1%) that were used in this test. The effect of triamcinolone acetonide under these conditions was particularly graphic in that most of the animals could not survive the testing procedure and experienced extreme weight loss and ultimately death.

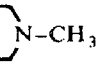
Finally, the diffusion of XVI through hairless mouse skin was measured over 24 h. During that time XVI delivered significantly more hydrocortisone through the skin than hydrocortisone itself. It is significant that, although the half-life of XVI in 10% plasma was about 6.4 h [2] there was no intact XVI found in the receptor phase of the diffusion cells. Thus, in addition to nearly doubling the amount of hydrocortisone delivered through the skin, XVI delivered only hydrocortisone which substantiated the suggestion that XVI was indeed a prodrug of hydrocortisone at least from a systemic viewpoint. This idea is supported by the similarity in systemic toxicity exhibited by XVI and hydrocortisone. Although these data cannot establish whether the activity exhibited by XVI locally is due to XVI or hydrocortisone, the significant difference in local toxicity may suggest that the former is true.

The use of amidocarboxylic acid esters of steroids has been shown to increase the delivery of hydrocortisone through skin in at least one case (XVI) and to result in decreased local toxicity but similar systemic toxicity compared to hydrocortisone. The use of these esters as derivatives of the more potent 17α-esters, on the other hand, is complicated by the fact that the 17α-esters are not prodrugs [3] but are active themselves (Raab and Gmeiner, 1976). However, one of the mixed esters (XXVI) was found to exhibit less systemic toxicity than its parent 17α-ester while retaining a portion of the local activity of the parent drug. Thus, this approach has led to

[2] Unpublished results, Sloan and Bodor.
[3] Hydrocortisone-17α-valerate and -17α-butyrate diffuse through hairless mouse skin intact while the -17α-propionate is half-hydrolyzed to hydrocortisone under these conditions (unpublished results, Sloan and Bodor).

TABLE 7



Compound	R	R'	Comments
I	H	H	Hydrocortisone
Hydrocortisone esters			
II	$COCH_3$	H	Hydrocortisone-21-acetate
III	H	COC_2H_5	Hydrocortisone-17α-propionate
IV	H	COC_3H_7	Hydrocortisone-17α-butyrate
V	H	COC_4H_9	Hydrocortisone-17α-valerate
Hydrocortisone-21-succinamate esters			
XV	$CO(CH_2)_2CON(CH_3)_2$	H	
XVI	$CO(CH_2)_2CON(C_2H_5)_2$	H	
XVII	$CO(CH_2)_2CON(C_3H_7)_2$	H	
XVIII	$CO(CH_2)_2CON(C_4H_9)_2$	H	
XIX	$CO(CH_2)_2CON(C_6H_{13})_2$	H	
XX	$COCH_2OCH_2CON(C_2H_5)_2$	H	
XXI	$CO(CH_2)_2CON$ 	H	
XXII	$CO(CH_2)_2CON$ 	H	
XXIII	$CO(CH_2)_2CON$ 	H	
Hydrocortisone-17α-ester-21-succinamate diesters			
XXIV	$CO(CH_2)_2CON(C_2H_5)_2$	COC_2H_5	
XXV	$CO(CH_2)_2CON(C_2H_5)_2$	COC_3H_7	
XXVI	$CO(CH_2)_2CON(C_2H_5)_2$	COC_4H_9	
Miscellaneous			
XXVII	$CO(CH_2)_2CON(C_2H_5)_2$		11-ketone, Δ^1
XXVIII	$CO(CH_2)_2CON(C_2H_5)_2$		9α-fluoro, Δ^1
XXIX	H	$CO(CH_2)_2CON(C_2H_5)_2$	
XXX		H	
XXXI		H	
XXXII		H	

increased delivery of hydrocortisone and decreased local toxicity in one case (XVI), and decreased systemic toxicity in another (XXVI) compared to hydrocortisone while at the same time giving derivatives of hydrocortisone that are more active than hydrocortisone or hydrocortisone-21-acetate topically.

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