Corp., and are within 0.4% for C, H, and N.

General Method for the Preparation of α-Arylmalonates. A solution of the ethyl arylacetate (100 mmol) in diethyl carbonate (50 mL) was added dropwise to a stirred slurry of NaH (150 mmol) in diethyl carbonate (100 mL). The suspension was stirred at room temperature overnight, poured onto ice–water, and extracted with ether (3 × 150 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a colorless oil, which was distilled to give the pure diethyl α-arylmalonate. Yields and physical constants are shown in Table II.

Diethyl 1*H*-Phenalene-2,2(3*H*)-dicarboxylate (7). A solution of diethyl malonate (16 g, 100 mmol) and **6** (31 g, 100 mmol) in ethanol (600 mL) and THF (100 mL) was added to a solution of sodium ethoxide (210 mmol) in ethanol (300 mL) at 0 °C. After 4 h, the mixture was allowed to warm to ambient temperature and was stirred for 5 h. The resulting mixture was concentrated, diluted with water, and extracted with ether (3 × 200 mL). The combined extracts were dried and evaporated to give a brown oil, which was purified by chromatography [silica gel (50:1) using 10% ethyl acetate/hexene as the eluting solvent] to give 30.0 g (97%) of a clear viscous oil: NMR δ 2.7 (m, 6 H), 6.1 (q, 4 H), 6.55 (s, 4 H), 9.1 (t, 6 H); mass spectrum, *m/e* (100%) 310 (M^+).

General Method for the Preparation of 4-Substituted Isoxazolidine-3,5-diones. An ethanol solution of the malonate (50 mmol) was added to a solution of hydroxylamine (75 mmol) and sodium ethoxide (75 mmol) in ethanol (100 mL) at 0 °C. The resulting solution was stirred for 8 h at 0 °C and then for 15 h at ambient temperature. The resulting solution was concentrated and acidified with 0.5 M HCl at 0 °C. Solids were collected by filtration and dried in vacuo. Oils were extracted with methylene chloride (3 × 100 mL) and were converted to their sodium salts with sodium ethoxide in ethanol. Compounds were recrystallized from ethanol/ether to a constant melting point. Yields and physical constants are shown in Table I.

Relative Affinity of 17α- and/or 21-Esters and 17α,21-Diesters of Cortisol for a Glucocorticoid Receptor from Rat Thymocytes

A. J. Solo,* Kenneth M. Tramposch, Daniel W. Szeto, and Mark J. Suto

Department of Medicinal Chemistry, State University of New York at Buffalo, Amherst, New York 14260.
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The affinity, relative to cortisol (1), of 17α- and 21-esters and 17α,21-diesters of cortisol for the glucocorticoid receptor of rat thymus cytosol was determined by a competitive binding assay which used [^{3}H]dexamethasone. Esterification of the 21-hydroxy group of cortisol caused a loss of relative affinity to 0.046 for acetate and 0.32 for valerate. Esterfication of the 17α-hydroxy group resulted in an increase in relative affinity to 1.14 for acetate, 12.4 for butyrate, and 11.5 for valerate. Diesters had relative affinities which reflected both trends. Thus, the 21-acetate, 21-propionate, 21-butyrate, and 21-valerate of cortisol 17-acetate had relative affinities of 0.036, 0.093, 0.152, and 0.272. The 21-acetate, 21-propionate, and 21-butyrate of cortisol 17-valerate had relative affinities of 0.76, 1.17, and 1.33.

In this paper we report the effect of esterifying the 17α- and/or 21-hydroxy groups of cortisol on the relative affinity of these compounds for a glucocorticoid receptor. Cortisol esters are of interest to us because cortisol is the prototype glucocorticoid and because several of its esters have proven utility as drugs.[1]

Previous studies of glucocorticoids have shown that esterification of the hydroxy groups of the side chain can affect biological activity, and the effect is especially great if the compounds are administered topically.[2-11] Some correlation between the physical properties of glucocorticoids and their topical activity has been observed,[2-12] but the correlation is inadequate to account fully for the activity of these compounds. Increased lipophilicity is expected to enhance the ability of a steroid to penetrate the skin, and, in the vasoconstrictor assay, steroidal esters typically show higher activity than steroidal alcohols.[3,11,12] If the acyl moiety of a steroidal ester becomes too large, vasoconstrictor activity diminishes.[3,7,9] This is not surprising in that many biological properties have been shown to have a nonlinear dependence on lipophilicity.[13,14] However, when the combined data for topical antiinflammatory activity[5] and vasoconstrictor activity[3,7] for steroidal 17α,21-diesters are examined, it is revealed that these data are not fully explained even by assuming nonlinear dependence on their calculated π values. Among the many feasible explanations for these data is the possibility that additional factor(s), such as differential effects of structure on receptor affinity, must be invoked. While further study of any of the factors which may modulate the biological activity of glucocorticoids could aid in the design of improved hormones or antihormones,[15-19] we were particularly

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Table I. Cortisol Esters: Characterization and Relative Binding Affinity

cortisol ester	solvent[a]	mp, °C	lit. mp, °C	yield	formula[b]	rel binding affinity[c]
cortisol						1
17-acetate	A-P	232-234	234-237[d]	82		1.14
17,21-diacetate	A-H	211-213	216-217[d]	70	$C_{25}H_{34}O_7$	0.036
17-acetate, 21-propionate	A-H	205-207		63	$C_{26}H_{36}O_7$	0.093
17-acetate, 21-butyrate	A-P	131-133		67	$C_{27}H_{38}O_7$	0.152
17-acetate, 21-valerate	A-H	160-162		85	$C_{28}H_{40}O_7$	0.272
17-butyrate	A-H	208-210	208-210[d]			12.4
17-valerate		159.5-160.5	159-161[d]			11.5
17-valerate, 21-acetate		foam		73	$C_{28}H_{40}O_7$	0.76
17-valerate, 21-propionate		foam		85	$C_{29}H_{42}O_7$	1.17
17-valerate, 21-butyrate	A-P	141-142		51	$C_{30}H_{44}O_7$	1.33
21-acetate	A-H	218-220	219-220[e]			0.046
21-valerate	A-H	193-195			$C_{26}H_{38}O_6$	0.32

[a] A = acetone; P = petroleum ether; H = hexane. [b] All compounds whose formulas are given analyzed for C and H within ±0.4% of theoretical value. [c] Binding affinity for the dexamethasone receptor of rat thymus cytosol measured relative to cortisol = 1. [d] Reference 34. [e] Reference 35.

interested in studying hormone–receptor interactions because of the potential utility of such data in designing agents to alkylate the glucocorticoid receptor(s).[20,21]

A number of putative glucocorticoid receptors have been studied. We chose to use the dexamethasone receptor of rat thymus because its isolation is well defined,[21,23] it has been extensively studied as a model glucocorticoid receptor,[21,23] and thymolytic activity classically has been used as a measure of glucocorticoid activity.

Chemistry. Steroidal 17α-esters required for this study were synthesized by hydrolysis at pH 3 of the appropriate cyclic 17,21-orthoesters.[24] The cyclic orthoesters were prepared by interchange with simple orthoesters.[25] Esters at the 21 position were introduced by acylation with the appropriate acid anhydride in pyridine.

Competitive Binding Studies. Several groups have measured the relative affinity of nonradioactive steroids for glucocorticoid receptors by using [^{3}H]dexamethasone in a competive assay.[23,26,27] In this study we employed a variant[28] of Rosen's assay[29] in which the animals were not adrenalectomized. To minimize the effect of biological variables, we chose to measure the relative ability of the steroidal esters to inhibit [^{3}H]dexamethasone binding to the glucocorticoid receptor of rat thymus.

Cytosol from rat thymuses was pooled and freed from endogenous steroids by treatment with charcoal. Aliquots of this cytosol were incubated with [^{3}H]dexamethasone, [^{3}H]dexamethasone plus 100-fold excess of unlabeled dexamethasone, and [^{3}H]dexamethasone plus 1 to 100-fold excess of competitor. After unbound steroids were removed by charcoal, bound radiolabeled dexamethasone was determined by a scintillation counter. All of the above measurements were made in triplicate and were repeated on two or more batches of cytosol, and the results were averaged. The difference between the radioactivity bound by fractions containing only [^{3}H]dexamethasone and that retained by aliquots containing [^{3}H]dexamethasone plus a 100× excess of unlabeled dexamethasone measured specific binding. The difference between the amount of radioactivity bound in the presence of [^{3}H]dexamethasone and that bound in the presence of a competitor was divided by specific binding and multiplied by 100 to give percent specific binding. For each concentration of a competitive steroid studied, percent specific binding was plotted against the log of the concentrations of competitor. The curves were compared at the 50% specific binding level and referenced to cortisol, whic was assigned an arbitrary activity of 1. The results are summarized in Table I.

Discussion

The data in Table I reveal a dichotomy between the effect on receptor binding of esterifying the 17α- or 21-hydroxy group of cortisol. In all cases studied, acylation of the 17α-hydroxy group resulted in higher affinity for the receptor. The increase, which is modest for acetate, is similar for butyrate and valerate, with both of the latter derivatives having more than a tenfold increase in affinity relative to cortisol. In contrast, conversion of the 21-hydroxy group of cortisol or cortisol 17α-ester to an acetate results in a large loss in binding affinity. As one proceeds up the homologous series from acetate to valerate, the loss in binding affinity is partially overcome, but none of the C-21 esters studied had an affinity as great as that of the corresponding C-21 hydroxy compound.

These experiments establish that esterification of the C-17α- and/or C-21 hydroxy groups of a steroid can affect its affinity for a glucocorticoid receptor. The stability of these esters when applied topically has not been established. If they could be shown to reach the receptor site(s) intact, then the effect of esterification on receptor affinity would have to be considered as one of many factors affecting the topical activity of glucocorticoids.[30] Currently,

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we are exploring the possibility that the increased binding affinity of selected glucocorticoid esters may be used to develop antiglucocorticoids[15-19,32] or compounds capable of alkylating glucocorticoid receptor site(s).

Experimental Section

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. IR spectra were determined on a Beckman IR-8 or a Perkin-Elmer 297 spectrometer. NMR spectra were determined on a Varian A-60 or on a Varian T-60 spectrometer in $CDCl_3$ with a Me_4Si internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. All compounds synthesized or submitted to the competitive binding assay appeared to be pure as shown by TLC and showed the expected IR and NMR spectra.

Trimethyl orthobutanoate[33] was synthesized from butyronitrile. The known 11β,17α,21-trihydroxy-4-pregnene-3,20-dione 17-acetate was synthesized from cortisol essentially according to the procedure of Gardi, Vitali, and Ercoli[25] but substituting triethyl orthoacetate for trimethyl orthoacete. Similarly, 11β,17α,21-trihydroxy-4-pregnene-3,20-dione 17-butanoate[34] was synthesized from cortisol. The 11β,17α,21-trihydroxy-4-pregnene-3,20-dione 17-pentanoate was a gift from Westwood Pharmaceuticals, Inc., Buffalo, NY.

Conditions for Esterifying the C-21 Hydroxy Group of Cortisol and of Cortisol 17-Esters. Cortisol or the appropriate cortisol 17-ester (500 mg, 1.1–1.2 mmol) was dissolved in 1 mL of pyridine and 1 mL of acetic, propionic, butyric, or valeric anhydride. The solution was allowed to stand at room temperature overnight. Water was added, and the mixture was extracted with CH_2Cl_2 twice. The organic extracts were combined, washed twice with 10% HCl, dried ($MgSO_4$), and concentrated to dryness under vacuum. The residue was chromatographed on 100 g of silica gel, eluting with $CHCl_3$–acetone. The esters obtained are listed in Table I.

Competitive Binding Study. In a typical experiment, six male Sprague-Dawley rats weighing 80–100 g were decapitated, and the thymus glands were exercised and freed of blood and connective tissue. The pooled thymuses were weighed, and a volume (100 mL/1 g of tissue) of TED buffer (0.01 M Tris, 0.0015 M EDTA, 0.001 M dithiothreitol, pH 7.4) containing 10% glycerol was added. The tissue was minced with a pair of scissors and homogenized in a glass–Teflon homogenizer at 4 °C (ten complete strokes of the motor-driven pestle were applied). The homogenate was centrifuged (Beckman LS-10 ultracentrifuge using an SW-30 rotor) for 1 h at 100000g, 4 °C, and the supernatant fraction (cytosol) was retained. The supernatant was treated with a charcoal suspension (1 g of activated charcoal, 50 mg of Dextran T-70 in 10 mL of TED buffer) that was 20% of the total volume of the supernatant and stirred at 4 °C for 10 min to remove any endogenous steroid. The mixture was centrifuged twice at 15000 rpm in a Sorvall refrigerated centrifuge at 4 °C to ensure removal of the charcoal.

Conical tubes (2 mL, polyethylene) containing 2×10^{-8} M (1,2-^{3}H)dexamethasone (Amersham, 25 C/mmol), 2×10^{-8} M [^{3}H]dexamethasone and 2×10^{-6} M cold dexamethasone, and 2×10^{-8} M [3H]dexamethasone and a 1- to 100-fold excess of cold test compound were allowed to incubate with 0.5 mL of the above cytosol preparation at 4 °C for 4 h. After the incubation period, the tubes were each treated with 100 μL of the above charcoal suspension, vortexed, and incubated for 10 min at 4 °C. The tubes were then centrifuged in a Beckman clinical centrifuge at 1800 rpm for 5 min at 4 °C. Two-hundred microliters of the supernatant from each tube was assayed for radioactivity in 7 mL of ACS scintillation fluid (Amersham) using a Packard Tricarb liquid scintillation counter. Each sample was counted for 5 min, and the raw counts per minute were used in the calculation of specific binding. Each experiment was run making triplicate determinations of competition. All experiments were repeated using a different pool of cytoplasm at least twice and in selected cases three times.

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Book Reviews

Amino Acids, Peptides and Proteins. Volume 11. Specialist Periodical Reports. By R. C. Sheppard, Senior Reporter. The Chemical Society, Burlington House, London. 1981. xxi + 552 pp. 13.5 × 22.5 cm. $131.50.

The latest volume in this continuing series covers the literature published during 1978 and continues to follow the time-honored style and format of its predecessors. The usual major topics are reviewed by the dedicated staff of "Reporters": amino acids (G. C. Barrett); structural investigations of peptides and proteins (R. Harrison, M. Rangarajan, and A. Dell); X-ray structure (W. D. Mercer); conformational interactions in solutions (R. H. Pain and eight other contributors); peptide synthesis (I. J. Galpin and M. F. J. Galpin); chemical structure and biological activity of hypothalamic releasing hormones (D. H. Coy), posterior pituitary peptides (M. Manning, M. Kruszinski, and W. H. Sawyer), pancreatic hormones (D. Brandenburg, D. Saunders, and B. E. Rudolph), gastrointestinal hormones (D. Gillesssen and R. O. Studer), vasoactive peptides (P. D. Roy), and the enkephalins and endorphins (P. W. Schiller); and, lastly, complexes of amino acids, peptides, and proteins (R. W. Hay and D. R. Williams). As in other volumes, reaction schemes and structural formulas are strategically placed in the text, tables are used generously, and an author index is provided. Sad to note is that purchasers of Volume 11 will have to pay almost exactly twice as much as they did for Volume 10, and *eight times* more than for Volume 6.

Staff

Biochemistry of Disease. Volume 8. Polyamines in Biology and Medicine. Edited by David R. Morris and Laurence J. Marton. Marcel Dekker, New York. 1981. xiv + 459 pp. 15 × 23 cm. $55.00.

Polyamines are multivalent ligands whose ubiquitous presence in plants, animals, and microorganisms signals their strategic participation in a variety of processes essential for cell growth and function. Putrescine, spermidine, and spermine are the most biologically significant members of the class of polyamines, and alterations in their metabolism are associated with a large number of both neoplastic and nonneoplastic clinical conditions. Research on the biology of polyamines has been remarkably active during