given with the antigen. If necessary, the compounds were first dissolved in a slight molar excess of sodium bicarbonate and then diluted into the antigen solution. Groups of five animals were used for all dose levels and control groups.

To quantitate the PCA test, the mean diameter of each spot was graphed as a function of the relative antiserum concentration. The line, fitted by the least-squares equation, was extrapolated to the value at "zero" antiserum concentration (base value). The following equation was then used to calculate the percent inhibition:

$$\% \text{ inhibn} = \left(1 - \frac{\text{diameter of drug} - \text{base value}}{\text{diameter of control} - \text{base value}}\right) \times 100$$

The statistical significance of the results was determined by the Student's $t$ test ($p < 0.05$). An inhibition of 15% was significant.

**Chemistry.** Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Each analytical sample was homogeneous by TLC and had IR, UV, and NMR spectra compatible with its structure. Combustion analysis for C, H, and N gave results within 0.4% of theory.

The procedures for the preparation of the reported compounds are listed as methods A–C and may be considered as general methods of preparation. The reported yields for the products obtained were not maximized.

**Method A. 2-Methoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (16b).** A mixture of 2-amino-5-methoxybenzoic acid (27.0 g, 161 mmol), 6-chloro-3-pyridinecarboxamide (25.0 g, 160 mmol), and ethanol (500 mL) containing 15 mL of concentrated HCl was heated at reflux for 24 h. The mixture was cooled to 0 °C, and the resultant solid precipitate was collected by filtration to give 34.0 g (69.5%) of crude hydrochloride salt, mp 286–294 °C dec. This material was recrystallized twice from pyridine to give the analytical sample: yield 7.20 g (16.7%); mp 329–333 °C dec.

**Method B. 2-Methoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carbonitrile (16a).** A solution of 1.2 L of pyridine, 300 mL of DMF, 7.46 g (39.4 mmol) of *p*-toluenesulfonyl chloride, and 7.45 g (27.6 mmol) of **16b** was heated at 100 °C for 42 h. The mixture was cooled and poured onto 4 L of ice/$H_2O$ and acidified to pH 1 with concentrated HCl. The solid that formed was collected: yield 5.0 g (72.2%); mp 273–280 °C dec. The analytically pure nitrile was obtained in 56.2% yield after one recrystallization from pyridine, mp 281–285 °C dec.

**Method C. 2-Methoxy-8-(1*H*-tetrazol-5-yl)-11*H*-pyrido[2,1-*b*]quinazolin-11-one (16).** A mixture of 3.00 g (12.0 mmol) of **16a**, $NaN_3$ (2.22 g, 34.2 mmol), $NH_4Cl$ (1.83 g, 34.2 mmol), and 250 mL of DMF was heated at 115 °C for 20 h. The mixture was cooled, poured onto 1.5 L of ice/$H_2O$, and acidified with concentrated HCl. The solid that formed was collected: yield 2.93 g (83%); mp 279–299 °C dec. The sample of analytical purity was obtained by recrystallization from pyridine: yield 2.25 g (64%); mp 302–304 °C dec.

**Acknowledgment.** The authors thank André Salama for his assistance and many stimulating discussions. We also thank Mrs. U. Zeek and Dr. R. C. Greenough for microanalyses and spectra and Dr. D. J. Herzig for the rat PCA determinations.

# Isoxazolidine-3,5-diones as Lens Aldose Reductase Inhibitors

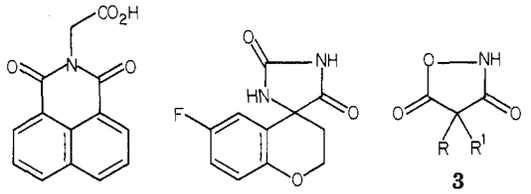
Allen B. Richon,*[†] Michael E. Maragoudakis,[‡] and James S. Wasvary[‡]

*Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, New Jersey 07901, and Ardsley, New York 10502.* *Received October 9, 1981*

Diabetes is a serious, debilitating disease which has several chronic complications, such as retinopathy, nephropathy, neuropathy, and cataracts.[1] The tissues involved in these complications have been shown to accumulate abnormally high amounts of sorbitol, the polyol which arises from the reduction of D-glucose by the enzyme aldose reductase. The pathogenic potential of sorbitol accumulation has been documented in the lens where elevated concentrations lead to osmatic swelling, resulting in disturbance in the metabolism and membrane transport mechanisms. Experimental models support the hypothesis that this contributes to the increased incidence of cataracts in human diabetes mellitus.[2-4]

The existence of aldose reductase in the aortic intima, brain, pancreas, and renal cortical tubules provides a pathogenic mechanism by which hyperglycemia can alter the metabolism and function of cells in these tissues via sorbitol accumulation. Therefore, an inhibitor of aldose reductase that is nontoxic and of appropriate duration of action in vivo may have value as a therapeutic agent against those diabetic complications in which sorbitol accumulation is thought to play a pathogenic role.[5]

The search for these agents showing activity as aldose reductase inhibitors has produced compounds such as alrestatin (1) and sorbinil (2). While clinical experience with aldose reductase inhibitors is limited, sorbinil remains one of the most promising aldose reductase inhibitors. The structural similarity of isoxazolidine-3,5-diones (3) to the spiro[chroman-4,4′-imidazolidine]-2′,5′-dione (2) has prompted us to explore isoxazolidine-3,5-diones for activity against aldose reductase.




1 2 3

## Results and Discussion

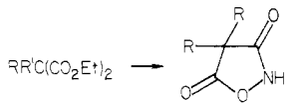
Compounds 8–17 were evaluated for activity in the rat lens aldose reductase assay. The percent inhibition of aldose reductase prepared from rat lenses was determined for each of the test compounds at a concentration of 50 μM by the procedure of Hayman and Kinoshita.[6] Compounds which inhibited partially purified aldose reductase

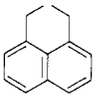
[†] Summit, NJ.
[‡] Ardsley, NY.

(1) D. Dvornik, *Annu. Rep. Med. Chem.*, 13, 159 (1978).
(2) D. Dvornik, N. Simard-Duquesne, M. Krami, K. Sestanj, K. H. Gabbay, T. H. Kinoshita, S. D. Varma, and L. O. Merola, *Science*, 182, 1146 (1973).
(3) S. D. Varma and T. H, Kinoshita, *Exp. Eye Res.*, 19, 577 (1974).
(4) S. D. Varma, A. Mizuno, and T. H. Kinoshita, *Science*, 195, 201 (1977).
(5) K. H. Gabbay, *N. Engl. J. Med.*, 288(16), 831 (1973).
(6) S. Hayman and J. H. Kinoshita, *J. Biol. Chem.*, 240, 877 (1965).

0022-2623/82/1825-0745$01.25/0 

Table I. Isoxazolidine-3,5-diones



| no. | R | R' | % yield | mp, °C | % inhibn of aldose reductase |
|---|---|---|---|---|---|
| 8 | $CH_3$ | 4-$AcNHC_6H_4$ | 72 | 136-137 dec | 16 |
| 9 | H | 2-pyridyl | 65 | 224 dec | 6.5 |
| 10 | H | 3-pyridyl | 53 | 258 dec | 37 |
| 11 | H | 2-$(CH_3O)C_6H_4$ | 70 | 135-137 | 0 |
| 12 | H | 3-$(CH_3O)C_6H_4$ | 65 | 119-121 | 75 |
| 13 | H | 2-$(CF_3)C_6H_4$ | 93 | 137-139 | 80 |
| 14 | H | $C_6H_5SOCH_2CH_2$ | 78 | 66-68 (Na salt) | 19 |
| 15 | H | $C_6H_5SO_2CH_2CH_2$ | 91 | 130 (Na salt) | 32 |
| 16 | H | $C_6H_5OCH_2CH_2$ | 57 | 87 | 35 |
| 17 |  | | 45 | 171-173 | 5.1 |

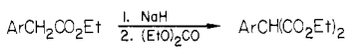
[a] Values for alrestatin, 87% at 50 μM; sorbinil, 82% at 10 μM.

Table II. Diethyl α-Arylmalonates

| Ar | % yield | bp (mm), °C | lit. bp (mm), °C |
|---|---|---|---|
| 2-pyridyl | 70 | 120-122 (0.35) | 130 (0.1)[a] |
| 3-pyridyl | 65 | 128-130 (0.3) | |
| 2-$(CF_3)C_6H_4$ | 60 | 134-135 (0.15) | 162-164 (4.5)[b] |
| 3-$(CH_3O)C_6H_4$ | 80 | 147-148 (0.5) | 150-151 (2)[c] |
| 3-$(CF_3)C_6H_4$ | 88 | 107-108 (0.3) | 145-150 (10)[d] |

[a] G. R. Newkome, J. M. Robinson, and N. S. Bhacca, *J. Org. Chem.*, **38**, 2234 (1973). [b] W. M. Lauer and L. I. Hansen, *J. Am. Chem. Soc.*, **61**, 3039 (1939). [c] H. Tsukamato, H. Yoshimura, and S. Toki, *Pharm. Bull. Jpn.*, **3**, 239 (1955). [d] *Chem. Abstr.*, 61 P13326*b* (1964).

Scheme I

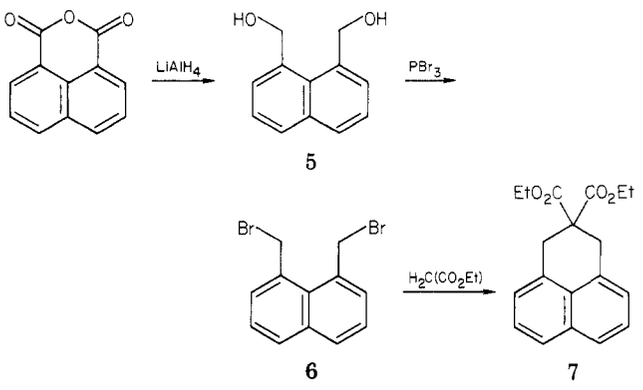


4

in the primary enzyme screen were retested for their effects on rat lenses ex vivo.[7,8] In vivo evaluation was performed according to the procedure of Dvornik[9] and Sweeley.[10]

The α-arylmalonates (**4**) in Table II were prepared via the enolate of the corresponding ethyl phenylacetate (Scheme I). Diethyl β-phenoxyethylmalonate was prepared by alkylation of the malonate anion.[11] Preparation of the oxo and dioxo derivatives of diethyl β-(phenylsulfinyl)ethylmalonate paralleled the synthesis of β-(phenylsulfinyl)ethylmalonic acid.[12] The diethyl *p*-acetamidophenylmethylmalonate was prepared as described from the corresponding diethyl *p*-nitrophenylmethylmalonate. Diethyl 1*H*-phenalene-2,2(3*H*)-dicarboxylate (**7**) was prepared from 1,8-naphthalic anhydride via the bis(hydroxymethyl)naphthalene **5**[13] and the bis(bromomethyl)naphthalene **6**[14] (Scheme II).

Scheme II



It can be seen from Table I that compounds **12** and **13** show good activity against partially purified aldose reductase. The failure of compounds **8**, **9**, **11**, **14**, and **17** to show useful activity is thought to result from the fact that their $pK_a$'s differ significantly from sorbinil's value of 7.85. Thus, their transport across membrane boundaries and binding to aldose reductase would differ significantly from the latter compound.

Although significant activity was demonstrated in vitro, only marginal activity could be detected with ex vivo and in vivo models. This suggests that while the proximity of an acidic carbonyl to an aromatic nucleus may be necessary for activity, compounds in this series must be modified such that their $pK_a$ range will reflect electronic charges within the molecule which will facilitate passage across ocular membranes at physiological pH. Studies are currently under way to investigate this hypothesis in the isoxazolidinone series.

## Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Spectral data were recorded for new compounds and were in agreement with the structure assigned. Elemental analyses were performed by the Microanalytical Laboratory, Pharmaceuticals Division, Ciba-Geigy

(7) J. H. Kinoshita, L. O. Merola, and S. Hayman, *J. Biol. Chem.*, **240**, 310 (1965).
(8) J. H. Kinoshita et al. *Invest. Ophthalmol.*, 8, 625 (1969).
(9) D. Dvornik, N. Simard-Duquesne, M. Krami, K. Sestanj, K. H. Gabbay, J. H. Kinoshita, S. D. Varma, and L. O. Merola, *Science*, **182**, 1146 (1973).
(10) C. C. Sweeley, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
(11) D. H. Peacock and P. Tha, *J. Chem. Soc.*, 2303 (1928).
(12) R. Pfister and H. Häfliger, *Helv. Chim. Acta.*, **44**, 232 (1961).
(13) R. E. Beyler and L. H. Sarett, *J. Am. Chem. Soc.*, **74**, 1409 (1952).
(14) E. D. Bergmann and J. Smuszkovicz, *J. Am. Chem. Soc.*, **75**, 2760 (1953).

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.*
*Received June 22, 1981*

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

(1) "AMA Drug Evaluations", 4th ed., American Medical Association, Chicago, 1980, pp 640–641 and 1026–1030.
(2) C. A. Winter and C. C. Porter, *J. Am. Pharm. Assoc.*, **46**, 515 (1957).
(3) A. W. McKenzie and R. M. Atkinson, *Arch. Dermatol.*, **89**, 741 (1964).
(4) D. I. Williams, D. S. Wilkinson, J. Overton, J. A. Milne, W. B. McKenna, A. Lyell, and R. Church, *Lancet*, 1, 1177 (1964).
(5) E. Shapiro, L. Finckenor, H. Pluchet, L. Weber, C. H. Robinson, E. P. Oliveto, H. L. Herzog, I. I. A. Tabachneck, and E. Collins, *Steroids*, **9**, 143 (1967).
(6) M. J. Busse, P. Hunt, K. A. Lees, P. N. D. Maggs, and T. G. McCorthy, *Br. J. Dermatol.*, 81(Suppl 4), 103 (1969).
(7) R. Gardi, R. Vitali, G. Falconi, and A. Ercoli, *J. Med. Chem.*, **15**, 556 (1972).
(8) A. Ercoli, G. Falconi, R. Gardi, and R. Vitali, *J. Med. Chem.*, **15**, 783 (1972).
(9) L. Toscano, G. Grisanti, G. Fioriello, L. Barlotti, A. Bianchetti, and M. Riva, *J. Med. Chem.*, **20**, 213 (1977).
(10) R. Vitali, S. Gladiali, G. Falconi, G. Celasco, and R. Gardi, *J. Med. Chem.*, **20**, 853 (1977).
(11) A. J. Shue, M. J. Green, J. Berkenkoph, M. Monahan, X. Ferandez, and B. N. Lutsky, *J. Med. Chem.*, **23**, 430 (1980).
(12) C. A. Schlagel, *J. Pharm. Sci.*, **54**, 335 (1965).
(13) C. Hansch, *Acc. Chem. Res.*, **2**, 234 (1969) and loc. cit.
(14) H. Kubinyi, *J. Med. Chem.*, **20**, 625 (1977) and loc. cit.
(15) A. Munck and C. Wira, *Adv. Biosci.* **7**, 301–330 (1971).
(16) G. Melnykovych and C. F. Bishop, *Endocrinology*, 88, 450 (1971).

0022-2623/82/1825-0747$01.25/0 