with four 20-mL portions of EtOAc. The combined EtOAc layers were drided over $MgSO_4$, filtered, and concentrated in vacuo to afford 150 mg (59%) of **44**. Two recrystallizations from IPA/IPE/hexane afforded 102 mg of pure **44**, mp 102–103 °C. TLC: silica gel, 1% CH_3OH/CH_2Cl_2, R_f 0.56. ^1H NMR ($CDCl_3$, 400 MHz): δ 4.19 (d, J = 4 Hz, 1 H), 4.12 (d, J = 4 Hz, 1 H), 3.63 (dd, J = 7, 11 Hz, 1 H), 3.63 (m, 1 H), 3.48 (t, J = 11 Hz, 1 H), 2.34 (t, J = 7 Hz, 2 H), 2.08 (m, 1 H, A of AB), 1.96 (m, 1 H, B of AB). ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ 178.7, 81.2, 78.4, 72.0, 61.1, 43.3, 40.7, 36.5, 33.9, 29.8, 29.2, 24.7. Anal. ($C_{14}H_{22}O_4$) C, H.

[1R-[1α,2β,3β,4α]]-7-[3-[(Hexyloxy)methyl]-7-oxabicyclo-[2.2.1]hept-2-yl]heptanoic Acid (47). To a solution of 120 mg (0.36 mmol) of **1** in 6 mL of EtOAc under Ar was added 24 mg of 10% Pd/carbon. The argon was replaced by hydrogen with several vaccum–fill cycles. The reaction mixture was stirred at room temperature under a slight positive pressure of hydrogen for 14 h. The catalyst was filtered off and the filtrate concentrated in vacuo to give 110 mg of crude product. Analysis of the crude product by 270-MHz ^1H NMR revealed the presence of 5–7% of **1**. The material was again subjected to the reaction conditions described above to afford 110 mg of **47**. Purification was effected by flash chromatoraphy on 22 g of silica gel with 2% CH_3OH/CH_2Cl_2 as eluant to afford 105 mg (87%) of **47**. TLC: silica gel, 8% CH_3OH/CH_2Cl_2, R_f 0.74, iodine. $[\alpha]_D$: −3.1° (c 1.37, $CHCl_3$). ^1H NMR ($CDCl_3$, 270 MHz): δ 4.38 (d, J = 5 Hz, 1 H), 4.23 (d, J = 5 Hz, 1 H), 3.3–3.1 (m, 4 H), 2.31 (t, J = 7 Hz, 2 H), 1.89 (m, 1 H), 0.87 (t, J = 7 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ 179.1, 80.1, 79.1, 71.2, 69.8, 47.0, 46.4, 34.0, 31.6, 29.7, 29.6, 29.3, 29.2, 29.0, 27.6, 25.8, 24.6, 22.6, 14.0. Anal. ($C_{20}H_{36}O_4$) C, H.

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Antiinflammatory and Aldose Reductase Inhibitory Activity of Some Tricyclic Arylacetic Acids[1]

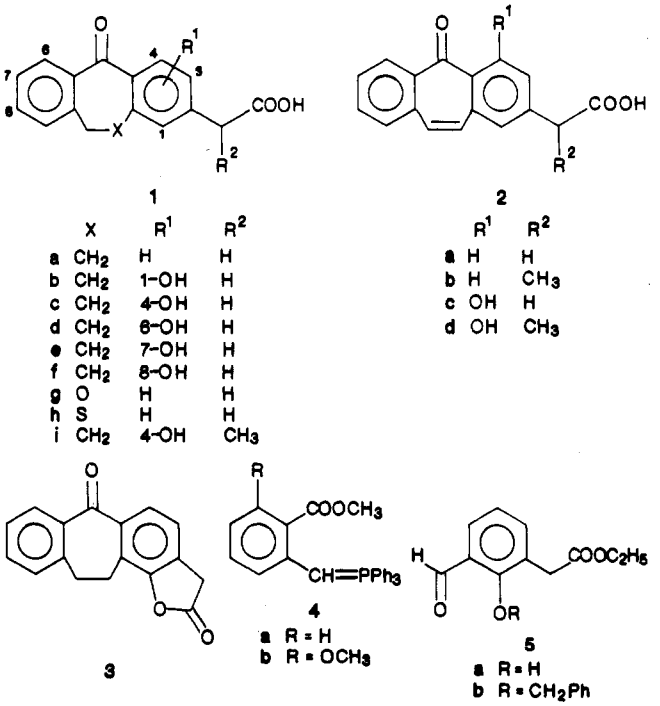
Mary Jane Cerelli,[†] Daniel L. Curtis,[‡] James P. Dunn,[‡] Peter H. Nelson,[‡] Tina M. Peak,[†] and L. David Waterbury*[†]

Institute of Pharmacology and Metabolism and of Organic Chemistry, Syntex Research, Stanford Industrial Park, Palo Alto, California 94304. *Received April 25, 1986*

A number of dibenztropone, dibenzsuberone, dibenzoxepin, and dibenzthiepin acetic acids were synthesized and tested for antiinflammatory/analgesic activity and also for their ability to inhibit rabbit lens aldose reductase (AR). It was found that the structural requirements for antiinflammatory/analgesic activity, believed to be mediated by inhibition of cyclooxygenase, were much more stringent than were those for AR inhibition. For example, the introduction of a hydroxyl group into positions 1, 4, 6, 7, or 8 on dibenzsuberone-2-acetic acid (**1a**) had relatively little effect on AR inhibition, but caused wide variations in antiinflammatory/analgesic activity.

The enzyme aldose reductase (AR) catalyzes the formation of sugar alcohols from sugars and has been implicated in the development of cataracts in diabetes and galactosemia[2,3] and in other complications of diabetes such as neuropathy and retinopathy.[4,5] Several compounds of diverse structure are known to inhibit the enzyme aldose reductase.[6–10] Recent reports that certain antiinflammatory drugs such as indomethacin, sulindac,[11–13] and aspirin[14] were inhibitors of the enzyme prompted us to present our findings that certain tricyclic arylacetic acids, in addition to having antiinflammatory and analgesic activity, are also potent inhibitors of the enzyme aldose reductase. The basis of this study was twofold. In the first place there was the possibility that some of the compounds tested may be more effective than the known inhibitors of the enzyme, and the additional activity in preventing prostaglandin synthesis may be useful in diabetic patients.[15,16] Secondly, a comparison could be made between a number of compounds with known antiinflammatory activity and possible aldose reductase inhibitory activity to determine any correlation.

Chemistry. The syntheses of a number of the compounds have been described previously (see Experimental Section). The 1- and 6-hydroxy compounds **1b** and **1d** were obtained by similar syntheses, the key step in each of which was a Wittig reaction between an appropriately substituted (2-carbomethoxybenzylidene)triphenylphosphorane (**4**) and a substituted benzaldehyde. Thus,



reaction between the ylid (**4a**), generated by reaction of the phosphonium bromide with ethanolic sodium ethoxide,

[†] Institute of Pharmacology and Metabolism.
[‡] Institute of Organic Chemistry.

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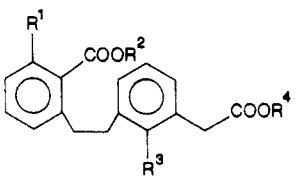
Table I. Biological Data

compd	rat paw assay (phenylbutazone = 1)	mouse writhing assay (aspirin = 1)	% inhibn of aldose reductase at given concn, M			IC_{50}
			10^{-4}	10^{-5}	10^{-6}	
1a	7 (54)[a]	1.5 (40)[b]	92	85	41	1.6×10^{-6}
1b	15 (60)[a]	~8 (24)[b]	95	95	84	5.0×10^{-8}
1c	~20 (96)[a]	4 (40)[b]	88	83	48	1.1×10^{-6}
1d	<0.2 (12)[a]	≥0.4 (8)[b]	NT[e]	78	30	2.8×10^{-6}
1e	<0.2 (12)[a]	~0.2 (8)[b]	96	91	54	8.3×10^{-7}
1f	~0.4 (30)[a]	~0.6 (16)[b]	96	89	49	1.0×10^{-6}
1g	~15 (120)[a]	4 (88)[b]	97	94	70	3.3×10^{-7}
1h	40 (384)[a]	14 (20)[b]	97	94	59	7.0×10^{-7}
1i[f]	~60 (66)[a]	4 (40)[b]	96	78	35	2.1×10^{-7}
2a	6 (84)[a]	2 (20)[b]	100	90	50	1.0×10^{-6}
2b[d]	68 (143)[a]	20 (30)[b]	90	74	28	3.1×10^{-6}
2c	5 (12)[a]	~1 (8)[b]	96	94	57	7.6×10^{-7}
2d	~30 (54)[a]	~6 (16)[b]	96	77	29	2.7×10^{-6}
3	3 (30)[a]	~1 (8)[b]	100	94	57	7.7×10^{-7}
sorbinil	NT	NT	95	95	80	6.7×10^{-8}
indomethacin[g]	16 (8–13)[c]	60 (100)	100	67	8	6.0×10^{-6}
ketoprofen	9 (54)	60 (42)	40	15	0	1.8×10^{-4}
salicylic acid	NT	NT	0	0	0	$>10^{-4}$
flufenamic acid	~6 (12)	<0.4 (32)	50	15	5	1.0×10^{-4}

[a] Number of rats. [b] Number of mice. [c] 95% confidence. [d] Isomer. [e] NT, not tested. [f] Racemic. [g] See ref 26.

and ethyl 2-benzyloxy-3-formylphenylacetate gave, after catalytic hydrogenation with concomitant debenzylation, an 82% overall yield of the diester (**6a**). Basic hydrolysis



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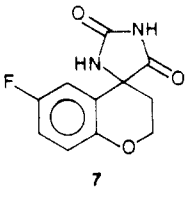
	R^1	R^2	R^3	R^4
a	H	CH_3	OH	C_2H_5
b	OCH_3	H	H	H
c	H	H	OH	H



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to the diacid, followed by polyphosphoric acid cyclization at 110 °C, then gave the tetracyclic lactone (**3**), which was easily hydrolyzed to the hydroxy acid (**1b**). The aldehyde (**5a**) was obtained by ortho-formylation of ethyl 2-hydroxyphenylacetate, using paraformaldehyde, tin(IV) chloride, and a tertiary base in toluene at 110 °C.[17] The yield in this reaction was low and was dependent on the amount of catalyst employed. The optimum yield, 19%, was obtained by using 0.05 mol of tin(IV) chloride/mol of substrate; both higher and lower proportions produced lower yields. Most of the unreacted starting material could be recovered during chromatography. The 6-hydroxy compound (**1d**) was obtained analogously, starting with a Wittig reaction between the ylid (**4b**) and lithium 3-formylphenylacetate. Hydrogenation of the intermediate stilbene, followed by ester hydrolysis, produced the diacid (**6b**), which could most efficiently by cyclized to the 6-methoxy tricyclic product by conversion to the acid chloride and reaction with aluminum chloride/nitromethane in dichloromethane. The 7- and 8-hydroxy acids were obtained by Arndt–Eistert homologation, followed by demethylation, of the corresponding 7- and 8-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-onecarboxylic acids.[18]

Results and Discussion

The compounds were tested for their ability to reduce carrageenan-induced inflammation of the rat paw, and analgesic activity was determined in the mouse phenylquinone writhing assay. They were also tested for inhibition of the reduction of glyceraldehyde to glycerol by rabbit lens aldose reductase (AR). Results are shown in Table I. Antiinflammatory and analgesic activity varied widely in the series as did the antiinflammatory/analgesic ratio. The ratio of potencies in these two assays varies considerably between different series of tricyclic compounds, despite the fact that cyclooxygenase-mediated conversion of arachidonic acid into prostaglandins is believed to be responsible for both effects. At a first approximation, however, and recognizing that the vagaries of absorption, distribution, and metabolism can effect in vivo antiinflammatory activity, the data in Table I can be regarded as a means of comparison of the abilities of the test compounds to inhibit cyclooxygenase and AR. It is apparent that structural requirements for antiinflammatory/analgesic activity are considerably more stringent than for inhibition of AR. Thus, all the test compounds

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showed IC_{50}'s of less than 10^{-6}, yet antiinflammatory potencies varied from about 60-fold (**1i** and **2b**) to 0 (**1a,e,f**). The effect of a hydroxyl substituent at five of the seven possible locations on the dibenzsuberone ring system can be seen in **1b–f**. All of these compounds were active inhibitors of AR with the 1-hydroxy isomer **1b** clearly the most potent, approximately equal to the reference compound sorbinil. This compound was also a more potent antiinflammatory agent than the nonhydroxylated compound **1a**, and the introduction of a 4-hydroxyl substituent (**1c**) also resulted in a considerable increase in antiinflammatory/analgesic potency. Apart from the potentiating effect at position 1, hydroxylation of the nucleus had little effect on inhibition of AR. In contrast, antiinflammatory potency was reduced ca. 15–30-fold by the introduction of a hydroxyl group at positions 6, 7, and 8 (**1a,e,f**). The introduction of a heteroatom into the seven-membered ring resulted in an increase in AR activity (**1g,h**), and this finding, coupled with the previously mentioned potentiating effect of the 1-hydroxyl substituent, may indicate the presence of a p-electron acceptor, close to a site which can accept a carboxyl group, at the AR active site. The slight potentiating effect of introduction of a double bond into the central ring (cf. **1a** and **2a**, **1c** and **2c**) also supports this postulate. A model of the AR active site has been proposed.[6,8] The site has planar lipophilic regions to which the benzene rings of the tricyclic compounds could bind and a charge transfer pocket appropriately located to accept the carbonyl group. Potentiation of activity caused by hydroxyl substituents led Kador et al. to postulate the presence of two hydrogen-binding sites at the edges of the planar region. It is noteworthy that compounds **1b,e,f**, which have hydroxyl groups near the appropriate positions, are more potent AR inhibitors than the unsubstituted molecule **1a**. These findings lend support to the proposed active-site model. Introduction of either oxygen or sulfur into the seven-membered ring (**1g,h**) caused a potentiation of antiinflammatory activity. The propionic acids **1i**, **2b**, and **2d** were the least active inhibitors of AR, yet were the most active antiinflammatory/analgesic agents. The lactone **3** showed lower potency in all three assays than did the 1-hydroxy acid **1b** from which it was derived. These compounds may well be interconvertible in vivo, with the open form **1b** presumably being the biologically active agent.

Since the foregoing observations indicate that the structural requirements for AR inhibition are not as stringent as for antiinflammatory/analgesic activity, it is perhaps surprising that the agents indomethacin, ketoprofen, and flufenamic acid, which are all inhibitors of cyclooxygenase, show virtually no AR inhibition at 10^{-6} M (see Table I). Ketoprofen, and to a lesser extent indomethacin, has many structural features in common with the series of compounds **1** and **2**. The most notable differences is that the flanking benzene rings in **1** and **2** are constrained to adopt a nonplanar conformation by the two-atom bridge in the central ring. Structural parallels between these compounds and sorbinil are less obvious, though sorbinil is a weak acid and clearly cannot adopt a planar configuration.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were taken as Nujol mulls on a Perkin-Elmer 710 B spectrometer, and frequencies are quoted to the nearest 5 cm^{-1}. NMR spectra were obtained in deuteriochloroform, using Me_4Si as an internal standard, on Varian A60, HA 100, and EM 390 and Bruker WM 300 instruments. Chemical shifts are quoted to the nearest 0.01 ppm relative to Me_4Si and coupling constants to the nearest 1 Hz.

The syntheses of **1a**,[19] **1c**, **1i**, **2c**, **2d**,[20] **1g**,[21] **1h**,[22] **2a**, and **2b**[23] have been described previously.

Ethyl 2-Benzyloxy-3-formylphenylacetate. Diisopropylethylamine (10.1 g, 0.078 mol) was added to a solution of ethyl 2-hydroxyphenylacetate (36 g, 0.2 mol) in toluene (400 mL). Tin(IV) chloride (2.58 g, 0.01 mol) was then added, and after 30 min paraformaldehyde (13.2 g). The mixture was heated at 96 °C for 6 h, then cooled, diluted with ether, and washed with dilute HCl. The organic solution was dried and evaporated, and the residue was chromatographed on silica gel (2 kg), eluting with 2000:100:1 to 2000:400:1 toluene–ether–AcOH to yield ethyl 3-formyl-2-hydroxyphenylacetate (7.9 g, 19%): mp 47–49 °C (ether–hexane); IR 1730, 1650, 1620 cm^{-1}; NMR δ 1.27 (d, 3 H, J = 7 Hz, CH_3), 3.69 (s, 2 H, CH_2), 4.19 (q, 2 H, J = 7 Hz, OCH_2), 7.00 (t, 1 H, J = 8 Hz, 5-H), 7.45–7.55 (m, 2 H, 4,6-H), 9.90 (s, 1 H, CHO). Anal. ($C_{11}H_{12}O_4$) C, H.

The aldehyde (7.9 g, 0.038 mol), K_2CO_3 (20 g, 0.145 mol), benzyl bromide (6.5 g, 0.038 mol), and DMF (50 mL) were stirred at 60 °C for 1.5 h. The solution was filtered and diluted with ether, then washed, dried, and evaporated to yield an oil which was filtered through a small plug of silica gel, eluting with ether, to afford the title compound (8.46 g, 75%): mp 40–43 °C (ether–hexane). Anal. ($C_{18}H_{18}O_4$) C, H.

(2-Carbomethoxybenzyl)triphenylphosphonium Bromide. Methyl *o*-toluate (100 g, 0.67 mol), *N*-bromosuccinimide (119 g, 0.67 mol), and dibenzoyl peroxide (0.25 g, 0.001 mol) were refluxed in CCl_4 (1500 mL) for 1 h. The cooled solution was filtered and evaporated. The residue was dissolved in MeCN (250 mL), and volatile components were again removed under vacuum so as to remove traces of CCl_4. The residue was dissolved in MeCN (1400 mL), and PPh_3 (201 g, 0.77 mol) was added. The solution was refluxed briefly, then cooled and diluted with ether so as to precipitate the title compound (233 g, 85%): mp 250–254 °C. Anal. ($C_{27}H_{24}BrO_2P$) C, H.

(2-Carbomethoxy-3-methoxybenzyl)triphenylphosphonium bromide was made analogously from methyl 2-methoxy-6-methylbenzoate[24] in 75% yield: mp 189–193 °C. Anal. ($C_{28}H_{26}BrO_3P$) C, H.

Ethyl 3-[2-(2-Carbomethoxyphenyl)ethyl]-2-hydroxyphenylacetate (6a). Na (0.97 g, 0.042 mol) was dissolved in EtOH (125 mL), and (2-carbomethoxybenzyl)triphenylphosphonium bromide (20.2 g, 0.042 mol) was added. After 3 min the ester (**5a**) (9.7 g, 0.034 mol) in EtOH (10 mL) was added. After 45 min the mixture was diluted with ether and washed with dilute HCl, then dried and evaporated. The residue was filtered through silica gel (400 g) eluting with 1:1 hexane–ether to remove Ph_3PO. The eluate was evaporated to yield the stilbene mixture (14.0 g), which was hydrogenated in a Parr apparatus at 60 psi for 12 h in EtOH (200 mL) containing 10% Pd/C (1.0 g). The solution was then filtered and evaporated, and the residue was recrystallized from ether–hexane to yield **6a** (9.4 g, 84%): mp 76–80 °C. Anal. ($C_{20}H_{22}O_5$) C, H.

3-[2-(2-Carboxyphenyl)ethyl]-2-hydroxyphenylacetic Acid (6c). The diester **6a** (9.4 g, 0.032 mol) was stirred in a solution of NaOH (6 g, 0.15 mol) in water (60 mL) at 60 °C for 2 h. The cooled mixture was then acidified to pH 4.0 with 2 N HCl. The resultant solution was extracted with EtOAc, and the extract was dried and evaporated to afford the diacid (7.2 g, 89%): mp 155–157 °C (EtOAc–hexane). Anal. ($C_{17}H_{16}O_5$) C, H.

1-Hydroxy-10,11-dihydro-5-oxo-5*H*-dibenzo[*a*,*d*]cyclo-

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hepten-2-ylacetic Acid Lactone (3). P_2O_5 (144 g) was added with stirring to 85% H_3PO_4 (85 mL) maintaining the temperature between 100 and 120 °C. The mixture was then kept at 115 °C for 2 h, and the diacid **6c** (7.4 g) was added. The reaction mixture was stirred at 115 °C for 75 min and was then poured into water. The product was extracted with EtOAc, and the extract was dried and evaporated to yield **3** (5.6 g, 86%): mp 180–183 °C (CH_2Cl–hexane); NMR δ 3.22 (s, 4 H, 10,11-H), 3.77 (s, 2 H, CH_2CO), 7.25–7.5 (m, 4 H, 3,7,8,9-H), 7.8–8.0 (m, 2 H, 4,6-H). Anal. ($C_{17}H_{12}O_3$) C, H.

1-Hydroxy-10,11-dihydro-5-oxo-5*H*-dibenzo[*a*,*d*]cyclohepten-2-ylacetic Acid (1b). The lactone **3** (7.0 g) was stirred in a mixture of water (250 mL) and 1.0 N NaOH (60 mL) at 60 °C until a clear solution was obtained (ca. 1 h). The solution was cooled, and the pH was adjusted to 4 by addition of 2 N HCl. The orange precipitate was removed by filtration and was recrystallized from EtOH (charcoal) to yield **1b** (5.36 g, 72%) as an off-white solid: mp 205–208 °C (aqueous dioxan); NMR (Me_2SO-d_6) δ 3.17 (br s, 4 H, 10,11-H), 3.63 (s, 2 H, CH_2CO), 7.12 (d, 1 H, J = 8 Hz, 3-H), 7.3–7.5 (m, 4 H, Ar H), 7.65 (dd, 2 H, J = 8 Hz, 6-H). Anal. ($C_{17}H_{14}O_4$) C, H.

3-[2-(2-Carboxy-3-methoxyphenyl)ethyl]phenylacetic Acid (6b). (2-Carbomethoxy-3-methoxybenzyl)triphenylphosphonium bromide (2.0 g, 0.0038 mol) was dissolved in DMF (20 mL), and 50% NaH in oil (365 mg, 0.0076 mol) was added. After 30 min lithium 3-formylphenylacetate (made by treatment of the acid with 1 equiv of aqueous LiOH, followed by drying under high vacuum at 70 °C (700 mg, 0.004 mol), in the minimum volume of DMF, was added. After 4 h at 25 °C, and brief heating to 80 °C, dilute Na_2CO_3 and ether were added. The aqueous solution was then acidified and extracted with EtOAc. The extract was dried and evaporated to give an oil (1.1 g), which was dissolved in EtOAc (30 mL) containing 10% Pd/C (250 mg) and hydrogenated at 60 psi for 36 h in a Parr apparatus. The solution was filtered and evaporated, the residue dissolved in MeOH (10 mL), and a solution of NaOH (2.0 g, 0.05 mol) in water (40 mL) added. The mixture was heated at reflux for 4 h, then cooled and washed with ether. The aqueous solution was acidified with dilute HCl and extracted with EtOAc. The extract was dried and evaporated, and the residue thus obtained was chromatographed on silica gel (50 g), eluting with 60:40:2 EtOAc–hexane–AcOH to produce **6b** (700 mg, 59%): mp 133–135 °C (ethyl acetate–hexane). Anal. ($C_{18}H_{18}O_5$) C, H.

Methyl 6-Methoxy-10,11-dihydro-5-oxo-5*H*-dibenzo[*a*,*d*]cyclohepten-2-ylacetate. The above diacid (1.0 g, 0.0032 mol) was dissolved in $CHCl_3$ (25 mL), and $SOCl_2$ (2 mL) and DMF (0.1 mL) were added. The reaction was heated at reflux for 1 h then evaporated to dryness. The residue was dissolved in dry C_6H_6 (10 mL), which was then removed under vacuum to remove traces of $SOCl_2$. The resulting diacid chloride was dissolved in CH_2Cl_2 (20 mL) and to it was added 6.0 mL of a solution of $AlCl_3$ (5.0 g) and CH_3NO_2 (3.03 mL) in CH_2Cl_2 (50 mL). The reaction mixture was kept at 25 °C for 3 days, and then aqueous NaCl (5 mL) was added. The organic phase was dried and evaporated, and to the residue was added THF (15 mL) and a solution of NaOH (0.5 g) in water (4 mL). After 2 h the mixture was washed with ether; then the aqueous solution was acidified and extracted with EtOAc. The crude product was treated with excess ethereal diazomethane, then chromatographed on silica gel, eluting with 4:1 hexane–EtOAc, so as to obtain the title compound (273 mg, 28%) as an oil.

6-Hydroxy-10,11-dihydro-5-oxo-5*H*-dibenzo[*a*,*d*]cyclohepten-2-ylacetic Acid (1d). The above product (250 mg, 0.0008 mol) was heated at reflux for 45 min in HOAc (2 mL) and 48% aqueous HBr (2 mL). The mixture was poured into water, and the product was extracted with EtOAc. This material was chromatographed on silica gel, eluting with 50:50:2 hexane–ethyl acetate–HOAc, to produce **1d** (105 mg, 47%): mp 149–151 °C (ethyl acetate–hexane); NMR δ 3.05 (br s, 4 H, 10,11-H), 3.60 (s, 2 H, CH_2CO), 7.18 (br s, 1 H, 1-H), 7.3–7.6 (m, 4 H, Ar H), 7.84 (d, 1 H, J = 9 Hz, 4-H). Anal. ($C_{17}H_{14}O_4$) C, H.

7-Hydroxy-10,11-dihydro-5-oxo-5*H*-dibenzo[*a*,*d*]cyclohepten-2-ylacetic Acid (1e). 7-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid (1.0 g, 0.0034 mol) was stirred at 25 °C in a mixture of $CHCl_3$ (10 mL), $SOCl_2$ (1 mL), and DMF (0.1 mL) for 2 h. Volatile compounds were then removed under vacuum, and the residue was dissolved in $CHCl_3$ (50 mL) and the solution was added to a large excess of ethereal diazomethane at 0 °C. After 2 h the solvents were removed to give the diazoketone (1.19 g): IR (Nujol mull) 2090 cm^{-1}. This material was dissolved in EtOH (30 mL), and the solution was heated to reflux. A solution of silver benzoate (80 mg) in triethylamine (1 mL) was then added, and nitrogen evolution ensued. After 2 min the mixture was cooled and filtered through celite. The eluate was evaporated, and the residue was chromatographed on silica gel, eluting with ether–hexane (1:1), to afford the ethyl ester of **1e** as an oil (0.66 g, 67% overall). This material was heated at reflux in MeOH (30 mL) and water (30 mL) containing KOH (0.25 g) for 1 h. The solution was cooled, acidified, and extracted with EtOAc. The extract was dried and evaporated, and the residue was recrystallized from acetone–hexane to afford 7-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-on-2-ylacetic acid (0.22 g, 38%): mp 112–114 °C. Anal. ($C_{18}H_{16}O_4$) C, H. This material was heated at reflux for 2 h in HOAc (2.5 mL) and 48% aqueous HBr (2.5 mL). The solution was cooled and diluted with water, and the product was extracted with EtOAc to afford **1e** (178 mg, 84%): mp 210–214 °C (aqueous MeOH); NMR δ 3.07 (br s, 4 H, 10,11-H), 3.42 (s, 2 H, CH_2CO), 6.8–7.5 (m, 5 H, Ar H), 7.85 (d, 1 H, J = 9 Hz, 4-H). Anal. ($C_{19}H_{18}O_4$) C, H. 8-Hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-on-2-ylacetic acid (**1f**) [mp 214–216 °C (EtOAc–hexane); NMR 3.04 (s, 2 H, 10,11-H), 3.58 (s, 2 H, CH_2CO), 6.63 (d, 1 H, J = 2 Hz, 9-H), 6.71 (dd, 1 H, J = 9, 2 Hz, 7-H), 7.15 (br s, 1 H, 1-H), 7.20 (dd, 1 H, J = 9, 2 Hz, 3-H), 7.81 (dd, 1 H, J = 9, 2 Hz, 6-H), 7.85 (d, 1 H, J = 9 Hz, 4-H). Anal. ($C_{19}H_{18}O_4$) C, H] was made in the same way in 22% overall yield from 8-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid.

Biological Assays. (a) Inhibition of Aldose Reductase. Aldose reductase was prepared from lenses of mature, New Zealand albino rabbits obtained frozen on dry ice from Pel-Freez Biologicals, Inc. The enzyme was isolated and assayed by methods similar to those employed by Hayman and Kinoshita,[25] who obtained the enzyme from calf lens. Batches of 50 lenses were thawed on ice and homogenized with 3 vol of distilled water, followed by centrifugation at 10000*g* for 15 min to remove insoluble material. Saturated ammonium sulfate was added to the supernatant to 40% saturation followed by recentrifugation 10 min later for 15 min at 10000*g*. Additional protein was removed by increasing the ammonium sulfate concentration to 50% and recentrifuging. Aldose reductase was precipitated from the 50% saturated solution by the addition of powdered ammonium sulfate to 75% saturation and was isolated by centrifugation.

The precipitated enzyme was assayed by following the UV absorbance at 340 nm with a Beckman DB-GT grating spectrophotometer attached to a recorder after dissolving the enzyme in 0.05 M sodium chloride and adding the other components to the cuvette. The enzyme (0.50 mL) was added to a quartz cuvette containing 0.2 mL of phosphate buffer (0.067 M, pH 6.0), 0.1 mL of NADPH (2×10^{-7} M, final concentration), 0.10 mL of the test drug, and 2.0 mL of water to a volume of 2.9 mL, and the cuvette was inserted into the spectrophotometer. Base readings were taken for 1 min. The reaction is started by the addition of 0.1 mL of D,L-glyceraldehyde (5×10^{-4} M, final concentration) to the cuvette and is followed by graphically determining the loss of absorbance at 340 nm for 2 min at 37 °C. The reaction rate observed was usually linear for at least 5 min. Drugs to be tested were dissolved in a minimal amount of *0.1 N NaOH* or other solvents and diluted with water so that 0.10 mL added to the cuvette would equal the final desired concentration. All results are shown in Table I as percent inhibition, i.e., based on a noninhibited control sample as 0%, and as IC_{50} values calculated by an iterative curve-fitting computer program. At 10^{-5} M, the following standard errors were calculated: compound **1a**, 85% ± 0.9% (n = 5); compound **1b**, 95% ± 0.9% (n = 5).

(b) Inhibition of Carrageenan-Induced Edema. This assay was carried out essentially as described in a publication[23] from these laboratories. Thus, 80–90-g female rats were given the test

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agent 1 h prior to the injection of carrageenan into one of the hind paws. The rats were sacrificed 4 h after administration of the drug, at which time both of the hind paws were excised and weighed separately. The potencies of the test agents relative to phenylbutazone were determined from dose–response plots of the percent increase in weight of the inflamed paw over the noninflamed paw. Usually at least three doses, using 6 rats/dose, were employed in constructing the plots.

(c) Inhibition of Phenylquinone-Induced Writhing. The assay was performed according to the procedure described in a publication[23] from these laboratories. Thus, 18–20-g male mice were given the test substance orally 20 min prior to an intraperitoneal injection of phenylquinone. The mice were observed for the next 10 min for writhing, and the potencies, relative to aspirin, were determined as in (b) using 8–10 mice/dose.

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Registry No. 1 (R_1 = OCH_3, X = CH_2, R_2 = H, methyl ester), 104156-24-3; **1a**, 54225-54-6; **1b**, 104156-09-4; **1c**, 72568-22-0; **1d**, 104156-10-7; **1e**, 104156-11-8; **1e** (ethyl ester), 104156-27-6; **1e** (R_1 = 7-OCH_3), 104156-28-7; **1e** (diazo ketone), 104156-26-5; **1f**, 104156-12-9; **1g**, 55689-65-1; **1h**, 61220-69-7; **1i**, 104156-13-0; **2a**, 58452-78-1; **2b**, 58452-80-5; **2c**, 72568-23-1; **2d**, 104156-14-1; **3**, 104156-15-2; **5a**, 104156-16-3; **5b**, 104156-17-4; **6a**, 104156-31-2; **6a** (stilbene, isomer 1), 104156-29-8; **6a** (stilbene, isomer 2), 104156-30-1; **6b**, 104156-22-1; **6b** (stilbene), 104156-20-9; **6b** (R_2 = CH_3), 104156-21-0; **6b** (diacid chloride), 104156-23-2; **6c**, 104156-18-5; 3-$OHCC_6H_4CH_2CO_2H$-Li, 104156-19-6; 2-$H_3CO_2CC_6H_4CH_2Br$, 2417-73-4; 2-$H_3CO_2CC_6H_4CH_2PPh_3^+Br^-$, 60494-73-7; 2-$HOC_6H_4CH_2CO_2CH_2CH_3$, 41873-65-8; 2-$H_3CC_6H_4CO_2CH_3$, 89-71-4; (2-carbomethoxy-3-methoxy benzyl)triphenylphosphonium bromide, 104172-27-2; methyl 2-methoxy-6-methylbenzoate, 79383-44-1; 7-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 64717-08-4; 7-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 104156-25-4; 8-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 64453-89-0; aldose reductase, 9028-31-3.

Furanose–Pyranose Isomerization of Reduced Pyrimidine and Cyclic Urea Ribosides

James A. Kelley,† John S. Driscoll,† John J. McCormack,‡ Jeri S. Roth,† and Victor E. Marquez*†

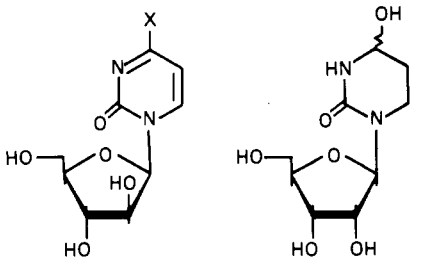
Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, and Department of Pharmacology, College of Medicine, and Vermont Regional Cancer Center, University of Vermont, Burlington, Vermont 05401. *Received December 11, 1985*

Tetrahydrouridine (THU, **2**) and other fully reduced cyclic urea ribofuranosyl nucleosides undergo a rapid, acid-catalyzed isomerization to their more stable ribopyranosyl form. This isomerization is characterized by a change in spectral properties and by a greater than 10-fold decrease in potency for those nucleosides that act as potent inhibitors of cytidine deaminase in their ribofuranose form. 1-(β-D-Ribopyranosyl)hexahydropyrimidin-2-one (**7**) was synthesized and used in conjuction with its furanose isomer **6** as a model compound for more extensive ^1H and ^{13}C NMR, mass spectral, and kinetic studies of this isomerization. The 0.4 δ upfield shift and 4-Hz increase in the $J_{1',2'}$ coupling constant for the pyranose anomeric proton in the ^1H NMR spectrum is indicative of a pyranose β-CI conformation in which the aglycon and C-2′ and C-4′ hydroxyls are equatorial. The mass spectra of trimethylsilylated pyranose nucleosides also show a characteristic large shift in the *m/z* 204–217 abundance and the appearance of two new rearrangement ions at M − 133 and M − 206. For furanose **6** the rate of isomerization is pH and temperature dependent with pyranose **7** predominating by a factor of 6–9 at equilibrium. At pH 1 and 37 °C, furanose **6** has an initial half-life of less than 12 min. Accordingly, this isomerization may explain the observed lack of enhanced *ara*-C levels in studies evaluating the oral administration of an *ara*-C and THU combination to species with an acidic stomach content.

Efficient in vivo inhibition of cytidine deaminase (CDA) has long been sought as a means of improving the therapeutic activity of the antitumor agent *ara*-C (**1a**) by overcoming its rapid deamination to inactive *ara*-U (**1b**).[1] The widespread distribution of CDA in many of the body's tissues, among them the liver, kidney, small intestine, and blood, ensures this rapid catabolism of *ara*-C and other cytidine analogues.[2] Moreover, significant CDA activity is encountered in human hematopoietic tissue as well as in acute and chronic myelogenous leukemia cells.[3,4]

One of the most potent and well-studied CDA inhibitors has been tetrahydrouridine (THU, **2**).[5] However, use of THU in combination with *ara*-C, both in animals and humans, has produced disappointing results for the most part. There has been little therapeutic advantage observed for this combination because of the parallel increase in toxicity associated with the resulting high plasma levels of *ara*-C.[6,7]

Use of intraperitoneal (ip) combinations of THU and *ara*-C against several in vivo mouse tumors, which were selected for their high levels of CDA activity, produced a significant increase in lifespan (ILS) against only the ascites form of S180J cells.[8] Results with other tumor lines



1a, X = NH_2
b, X = OH

2

†National Institutes of Health.
‡University of Vermont.

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