ether gave an oily precipitate which was separated, washed several times with ether, and dried under vacuum to give a light yellow powder identified as α-[Cp*Rh(17β-estradiol)][CF_3SO_3] (**8a**), while the supernatant phase gave the β-isomer **8b**. Overall yield (0.320 g, 79%) with α,β ratio 87:13. **8a**: (250 MHz, ^{1}H NMR, CD_3OD, δ ppm) 7.03 (d, J = 7.5 Hz, 1 H, H-1), 6.32 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 6.27 (d, 1 H, H-4), 0.8 (s, 3 H, Me-18), 2.09 (s, 15 H, Cp*). Anal. Calcd for $C_{30}H_{39}O_8F_6S_2Rh$: C 44.55, H 4.82; found: C 43.83; H 5.24. **8b**: (250 MHz, ^{1}H NMR, CD_3OD, δ ppm) 6.92 (d, J = 7.5 Hz, 1 H, H-1), 6.00 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 6.13 (d, J = 2.5 Hz, 1 H, H-4), 0.89 (s, 3 H, Me-18), 2.12 (s, 15 H, Cp*).

α-[Cp*Rh(3-*O*-(hydroxypropyl)-17β-estradiol)][BF_4]$_2$ (12a). As described for **8a**, [Cp*Rh(solvent)$_3$][BF_4]$_2$ was prepared in situ by mixing (0.16 g, 0.25 mmol) of [Cp*$RhCl_2$]$_2$ in acetone with $AgBF_4$ (0.200 g, 1 mmol) in THF solution. The resulting yellow solution was filtered into a new Schlenk tube. An amount of 0.165 g (0.5 mmol) of 3-*O*-(hydroxypropyl)estradiol in THF was added to this solution, and the reaction mixture was left for 2 h after which the light yellow solution was concentrated under vacuum. Addition of diethyl ether gave a creamy precipitate which was filtered, washed several times with diethyl ether, and then dried under vacuum. Identified as α-[Cp*Rh(3-*O*-(hydroxypropyl)-17β-estradiol)][BF_4]$_2$ (**12a**). Overall yield was 0.230 g, 62%. The supernatant phase gave the β-isomer **12b** (α,β ratio 87:13). **12a**: (250 MHz, ^{1}H NMR, CD_3COCD_3, δ ppm) 7.50 (d, J = 6.5 Hz, 1 H, H-1), 7.35 (dd, J = 6.5, 2.5 Hz, 1 H, H-2), 7.40 (d, J = 2.5 Hz, 1 H, H-4), 2.29 (s, 15 H, Cp*), 0.77 (s, 3 H, Me-18).

B. Receptor Binding Assays. Biochemicals, Materials, and Methods. Unlabeled steroids and protamine sulfate (from salmon grade X) were obtained from Sigma. [6,7-^{3}H]-17β-estradiol (52 Ci/mmol) was obtained from CEA Gif/Yvette, France.

Animal Tissues. Lamb uteri weighing approximately 7 g were obtained from the slaughterhouse at Mantes-la-Jolie, France. They were immediately frozen and kept frozen in liquid nitrogen prior to use.

Preparation of Lamb Uterine Cytosol. Lamb uteri were thawed and then minced. The resulting tissues were homogenized with an Ultra-Turrax in buffer A (0.05 M Tris-HCl 0.25 M sucrose, 0.1% β-mercaptoethanol, pH 7.4 at 25 °C). The homogenate was centrifuged at 105000g for 60 min in the 52 Ti rotor of a Beckman L5 ultracentrifuge. The protein concentration of the 105000g supernatent (cytosol) was determined by the method of Bradford.[24]

Protamine Sulfate Precipitation Assay. This technique was used for the separation of [^{3}H]-bound and free steroid.[8] Following incubation of aliquots of cytosol (200 μL containing 5 mg of protein/mL) with [^{3}H]estradiol, an equal volume of buffer containing 2.5 mg/mL protamine sulfate was added to each tube. The mixture was vortexed and allowed to stand at 0 °C for 10 min. The precipitate was filtered on glass fiber paper (Whatman GF/C) under light vacuum and washed with 40 mL of ice-cold buffer. The filter was then transferred to a scintillation vial and counted in 10 mL of ACS (Amersham). Radioactivity was measured in a LKB-1211 RackBeta with a counting efficiency of 30-40%.

Competitive Binding Assays. Aliquots of cytosol (200 μL) were incubated for 3 h at 0 °C with 2×10^{-9} M [^{3}H]estradiol in the presence or absence of competing unlabeled steroids (nine concentrations ranging from 1×10^{-10} to 1×10^{-6} M). Starting from a stock solution (10^{-3} M in absolute ethanol, serial dilutions of unlabeled steroids were performed in absolute ethanol just prior use. The percentage reduction in binding of [^{3}H]-labeled steroid (Y) was calculated by use of the logit transformation of Y.[8] The relative binding affinity (RBA) of the competitor is taken as the ratio of the concentrations of unlabeled estradiol/competitor required to inhibit half of the specific [^{3}H]-estradiol binding with the affinity of estradiol set at 100%.

Acknowledgment. We thank IRE-Medgenix (Belgium) and CNRS for financial support, T. Ojasoo for helpful discussion, and A. Cordaville for technical assistance.

(24) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248-254.

Synthetic Nonpsychotropic Cannabinoids with Potent Antiinflammatory, Analgesic, and Leukocyte Antiadhesion Activities

Sumner H. Burstein,*,† Charlene A. Audette,† Aviva Breuer,‡ William A. Devane,‡ Sharona Colodner,‡ Sharon A. Doyle,† and Raphael Mechoulam‡

Department of Biochemistry & Molecular Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655, and The Brettler Medical Research Center, Medical Faculty, The Hebrew University, Jerusalem 91120, Israel. *Received May 23, 1991*

Two strategies for the design of therapeutically useful cannabinoids have been combined to produce compounds with greatly increased antiinflammatory activity and with a low potential for adverse side effects. Enantiomeric cannabinoids with a carboxylic acid group at position 7 and with an elongated and branched alkyl sidechain at position 5′ have been synthesized and tested for antiinflammatory activity. They were effective when given orally at doses of 10 μg/kg in reducing paw edema in mice that had been induced by either arachidonic acid or platelet activating factor. Leukocyte adhesion to culture dishes was also reduced in peritoneal cells from mice in which the cannabinoids were orally administered in the same dose range as for the paw edema tests. Antinociception could be observed in the mouse hot plate assay; however, little stereochemical preference was seen in contrast to the above tests where the 3*R*,4*R* compounds are more active than the 3*S*,4*S* enantiomers. Finally, in agreement with earlier reports on the naturally occurring pentyl side chain acids, the synthetic acids showed little activity in producing catalepsy in the mouse, suggesting that they would be nonpsychtropic in humans.

Δ^1-Tetrahydrocannabinol (THC), the psychoactive component of marihuana, has been reported to exhibit activities in addition to its mood altering effects some of which may have therapeutic value.[1] Much effort has been expended over the years in seeking analogues that retain the properties of medicinal value and are devoid of the psychotropic effects. Novel approaches to this problem were recently reported by us. In one of them we showed

† University of Massachusetts Medical School.
‡ The Hebrew University.

(1) Mechoulam, R., Ed. *Cannabinoids as Therapeutic Agents*; CRC Press: Boca Raton, 1986.

0022-2623/92/1835-3135$03.00/0

that while the 1,1-dimethylheptyl (DMH) homologue of (3*R*,4*R*)-7-hydroxy-Δ^6-THC (**1a**, HU-210) is a very potent cannabimimetic, its enantiomer (**2a**, HU-211) lacks such activity.[2,3] However, **2a** is a potent antiemetic[4] and NMDA antagonist.[5] In a second approach to this problem, recently reported by us, it was shown that carboxylic acid derivatives of Δ^1- and Δ^6-THC may be of value in developing clinically useful cannabinoids.[6-8] The acids are believed to be free of psychoactivity in humans[9] and are inactive in animal behavioral tests.[10,11] These compounds are, however, at least as active as the parent substances in various antiinflammatory and analgesic assays. The mechanistic basis for these properties is not fully understood; however, it may be related to their ability to inhibit the production of proinflammatory eicosanoids from arachidonic acid. These cannabinoid acids may represent a new class of nonsteroidal antiinflammatory agents (NSAID).

In the present work we have tried to combine the two approaches. We prepared and tested for antiinflammatory activity THC-type 7-oic acids in both enantiomeric series. We have also made use of the earlier findings that elongation and branching of the alkyl side chain generally results in dramatic increases in potencies of other types of cannabinoids.[1-4] An important feature of the cannabinoids reported here is the likelihood that they will have few, if any, undesirable side effects.[12] This prediction is based on the large body of findings showing the relative absence of toxicities for cannabinoids in general other than CNS effects. Since the acids do not exhibit cannabimimetic activity, it is very likely that they would be safe drugs. The results presented suggest that our goal of obtaining cannabinoids with high antiinflammatory activity, apparently free of THC-type effects, may be achievable.

Scheme I

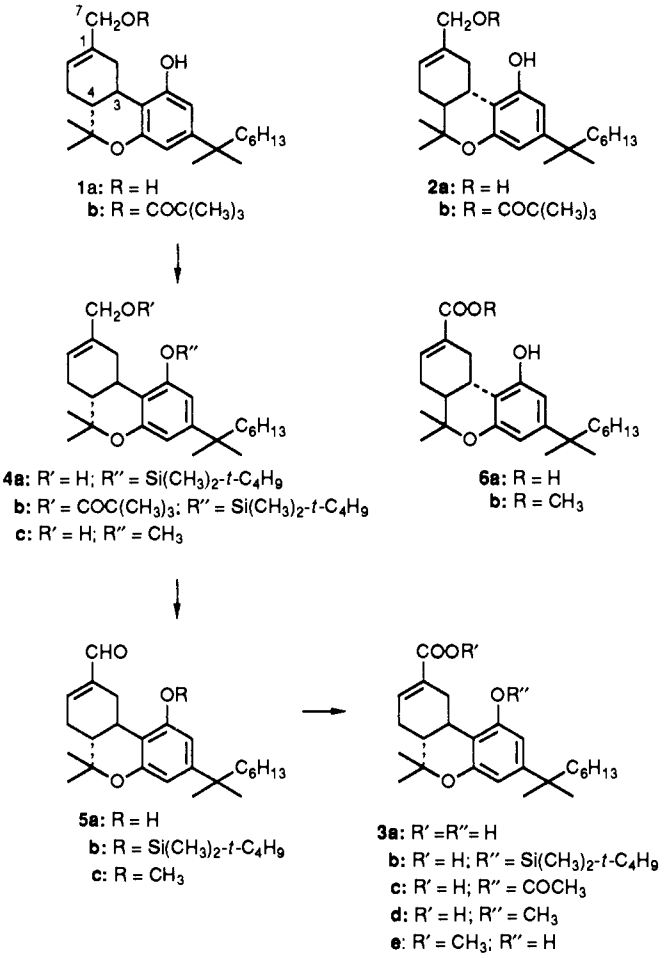

Table I. Inhibition of Arachidonic Acid-Induced Paw Edema[a]

dose (mg/kg)[b]	3a	6a
0.005	26.3 (28 ± 4)	–
0.010	52.6* (18 ± 6)	–
0.025	56.4* (17 ± 4)	–
0.050	73.7* (10 ± 4)	42.1 (22 ± 4)
0.100	97.4* (0.05 ± 11)	65.8* (13 ± 3)
0.250	100* (0.0 ± 5)	52.6* (18 ± 6)
0.500	100* (0.0 ± 6)	47.4* (20 ± 4)

[a] Values shown are percent inhibition of paw edema when compared to vehicle treated controls. Numbers in parentheses are the increases in paw volumes ± SE in microliters. Values marked with an asterisk (*) indicate 95% significance by ANOVA. $N = 5$ mice/group. For details see the Experimental Section. [b] Control mice were given peanut oil (50 μL) orally. Paw volume increase = 38 ± 4 μL.

Synthesis

We recently reported the synthesis of the individual, pharmacologically distinct 3*R*,4*R* and 3*S*,4*S* enantiomers of the 1,1-dimethylheptyl (DMH) homologues of 7-hydroxy-Δ^6-tetrahydrocannabinol (**1a** and **2a**, respectively).[13] We have now made use of the procedure for the preparation of the respective 3*R*,4*R* and 3*S*,4*S* acids. The starting material is a previously reported intermediate in

(2) Mechoulam, R.; Feigenbaum, J. J.; Lander, N.; Segal, M.; Järbe, T. U. C.; Hiltunen, A. J.; Consroe, P. Enantiomeric Cannabinoids: Stereospecificity of Psychotropic Activity. *Exper.* **1988**, 762–764.
(3) Little, P. J.; Compton, D. R.; Mechoulam, R.; Martin, B. R. Stereochemical Effects of 11-OH-Δ^8-THC-DMH in Mice and Dogs. *Pharmacol. Biochem. Behav.* **1989**, *32*, 661–666.
(4) Feigenbaum, J. J.; Richmond, S. A.; Weisman, Y.; Mechoulam, R. Inhibition of Cisplatin-induced Emesis in the Pigeon by a Non-psychotropic Synthetic Cannabinoid. *Eur. J. Pharmacol.* **1989**, *169*, 159–165.
(5) Feigenbaum, J. J.; Bergmann, F.; Richmond, S. A.; Mechoulam, R.; Nadler, V.; Kloog, Y.; Sokolovsky, M. Nonpsychotropic Cannabinoid Acts as Functional N-Methyl-D-Aspartate Receptor Blocker. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9584–9587.
(6) Burstein, S.; Hunter, S. A.; Latham, V.; Renzulli, L. Antagonism of Δ^1-THC Action by Its Metabolites. *Biochem. Pharmacol.* **1986**, *35*, 2553–2558.
(7) Burstein, S.; Hull, K.; Hunter, S. A.; Latham, V. Cannabinoids and Pain Responses; A Possible Role for Prostaglandins. *FASEB J.* **1988**, *2*, 3022–3026.
(8) Burstein, S.; Audette, C. A.; Doyle, S. A.; Hull, K.; Hunter, S. A.; Latham, V. Antagonism to the Actions of PAF by a Nonpsychoactive Cannabinoid. *J. Pharmacol. Exper. Ther.* **1989**, *251*, 531–535.
(9) Perez-Reyes, M. Pharmacodynamics of Certain Drugs of Abuse. In *Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs*; Barnett, G., Chiang, N. C., Eds.; Biomed. Pubs.: Foster City, 1985; pp 287–310.
(10) Mechoulam, R.; Ben-Zvi, Z.; Agurell, S.; Nilsson, I. M.; Nilsson, J. L. G.; Edery, H.; Grunfeld, Y. Δ^6-THC-7-oic Acid, A Urinary Δ^6-THC Metabolite: Isolation and Synthesis. *Exper.* **1973**, *29*, 1193–1195.
(11) Watanabe, K.; Yamamoto, I.; Oguri, K.; Yoshimura, H. Comparison in Mice of Pharmacological Effects of Δ^8-THC and Its Metabolites. *Eur. J. Pharmacol.* **1980**, *63*, 1–6.
(12) Burstein, S. Delta-1-THC-7-oic Acid and Analgesic and Antiinflammatory Agents. U.S. Patent 4,847,290, 1989; *Chem. Abstr.* **1990**, *112*, 677g.
(13) Mechoulam, R.; Lander, N.; Breuer, A.; Zahalka, J. Synthesis of the Individual, Pharmacologically Distinct, Enantiomers of a Tetrahydrocannabinol Derivative. *Tetrahydron: Asymmetry* **1990**, *1*, 315.

Table II. Inhibition of PAF-Induced Paw Edema[a]

treatment	dose (mg/kg)[b] 0.05	0.10	0.25	0.5	1.0	20.0
3a	101* (−1.2 ± 4)[c]	135* (−10 ± 3)[c]	150* (−14 ± 7)[c]	171* (−20 ± 5)[c]	–	–
6a	44.4 (10 ± 5)[c]	38.7* (19 ± 16)[d]	31.9* (21 ± 2)[d]	60.1* (12 ± 7)[d]	–	–
3e	–	39.3* (19 ± 6)[d]	63.9* (11 ± 3)[d]	76.0* (7.5 ± 10)[d]	–	–
6b	–	−44.2 (38 ± 7)[e]	7.6 (24 ± 4)[e]	−7.5 (28 ± 4)[e]	–	–
1a	–	–	–	6.3 (30 ± 6)[f]	56.3 (14 ± 5)[f]	–
2a	–	–	–	−37.5 (44 ± 8)[f]	21.8 (28 ± 5)[f]	–
Δ^6-THC-7-oic acid[g]		–	–	–	–	50.2

[a] Values shown are percent inhibition of paw edema when compared to vehicle treated controls. Numbers in parentheses are paw volume changes ± SE in microliters. Values marked with an asterisk (*) indicate 95% significance by ANOVA. $N = 8$ mice/group. For details see the Experimental Section. [b] Control mice were given peanut oil (50 μL) orally. Paw volume increases: *c*, 28 ± 7; *d*, 31 ± 3; *e*, 26 ± 4; *f*, 32 ± 7 μL. [g] See ref 27 for details.

Table III. Effects on Leukocyte Adhesion[a]

dose (mg/kg)[b]	1a	2a	3a	6a	Δ^6-THC-7-oic acid[c]
control	0.88 ± 0.08 (100)	0.88 ± 0.08 (100)	1.26 ± 0.05 (100)	1.26 ± 0.05 (100)	–
0.01	–	–	0.88 ± 0.03 (70)*	1.34 ± 0.14 (106)	–
0.05	1.12 ± 0.12 (127)*	1.09 ± 0.08 (124)*	1.34 ± 0.08 (106)	1.29 ± 0.05 (102)	–
0.10	0.94 ± 0.11 (106)	0.44 ± 0.03 (50)*	0.64 ± 0.08 (54)*	1.38 ± 0.17 (110)*	–
0.20	0.58 ± 0.06 (66)*	–	–	–	–
0.50	0.59 ± 0.05 (67)*	0.64 ± 0.06 (73)*	0.87 ± 0.08 (69)*	1.46 ± 0.05 (116)*	–
1.00	–	0.59 ± 0.06 (67)*	0.30 ± 0.03 (24)*	0.70 ± 0.12 (56)*	–
control	–	–	–	–	0.81 ± 0.03 (100)
20	–	–	–	–	0.67 ± 0.02 (82.7)*
40	–	–	–	–	0.55 ± 0.02 (67.9)*

[a] Values are the number of adhering cells × 10^6 ± SD. Numbers in parentheses are percent of control. For details see the Experimental Section. An asterisk (*) indicates 95% significance by ANOVA; otherwise not statistically significant. [b] Control mice were given 50 μL of peanut oil orally. Peritoneal cells were collected 90 min after oral administration of the cannabinoids. [c] See ref 25 for details.

the synthesis of **1a**, namely its 7-pivalate ester (**1b**).[13]

The phenolic group in **1b** was blocked as a dimethyl-*tert*-butylsilyl ether to give **4b**, which was reduced with lithium aluminum hydride to the free allylic alcohol **4a**. Oxidation of **4a** with chromic oxide in pyridine led to the aldehyde **5b**, which was further oxidized to the acid **3b** with sodium chlorite following a procedure used by Pellegata et al.[14] in the synthesis of oleuropeic acid. Removal of the phenolic protective group led to the desired acid **3a** (HU-239), which is best characterized as its acetate **3c**, mp 120–2 °C. Δ^6-THC-DMH-7-oic acid (**6a**, HU-235) in the 3*S*,4*S* series was synthesized following the same procedure, starting from the respective pivalate ester **2b**. A similar sequence of reactions has recently been used by Tius et al.[15]

The pentyl analogue of **3a**, which is a major metabolite of Δ^6-THC and is widely used as a hapten in the preparation of antigens for radioimmunoassay (RIA) of cannabinoids, has previously been synthesized by Mechoulam et al.[10] and Schwarz and Madan.[16] The corresponding Δ^1-THC-7-oic acid has also been prepared. (For a review, see Razdan.[17]) For more recent work see, Siegel et al.,[18] Huffman et al.,[19] Tius et al.,[15] and Baek et al.[20]

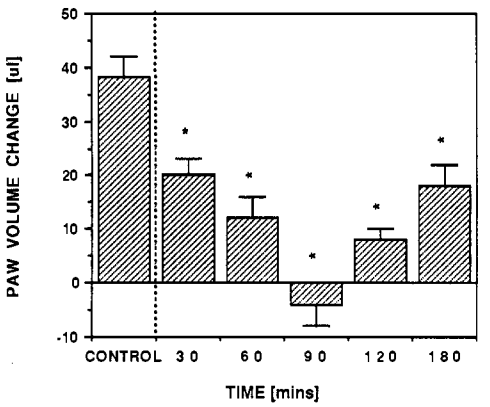

Figure 1. Effect of pretreatment time on arachidonic acid-induced paw edema by **3a**. The times shown are the intervals between the oral administration of **3a** (0.05 mg/kg) and the injection of arachidonic acid (1.0 mg/paw). For * 95% significance by ANOVA. $N = 5$ mice/group.

Pharmacological Results and Discussion

The induction of paw edema in rodents by injection of arachidonic acid has been used as an experimental model for inflammation.[21] Prior administration of NSAIDs in many cases leads to a dose-related inhibition of the edematous response which may be considered a predictor of

(14) Pellegata, R.; Ventura, P.; Villa, M.; Palmisano, G.; Lesma, G. An Improved Procedure for the Synthesis of Oleuropeic Acid. *Synth. Commun.* **1985**, *15*, 165.
(15) Tius, M. A.; Gu, X. Q.; Kerr, M. A. A Convenient Synthesis of (−)-11-Nor-delta-9-tetrahydrocannabinol-9-methanol. *J. Chem. Soc., Chem. Commun.* **1989**, 62.
(16) Schwarz, A.; Madan, P. A Convenient Synthesis of 11-Nor-delta-8-Tetrahydrocannabinol-9-Carboxylic Acid. *J. Org. Chem.* **1986**, *51*, 5463.
(17) Razdan, R. K. The Total Synthesis of Cannabinoids in *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; John Wiley & Sons Inc.; New York, 1981; Vol. 4., pp 186–262.
(18) Siegel, C.; Gordon, P. M.; Razdan, R. K. An Optically Active Terpenic Synthon for Delta-9-Cannabinoids: Synthesis of (−)-11-Hydroxy-delta-9-tetrahydrocannabinol (THC) and Its 1'1'-Dimethylheptyl Analogue. *J. Org. Chem.* **1989**, *54*, 5428.
(19) Huffman, J. W.; Zhang, X.; Wu, M. J.; Joyner, H. H. Regioselective Synthesis of (±)-11-Nor-9-carboxy-delta-9-THC. *J. Org. Chem.* **1989**, *54*, 4741.
(20) Baek, S. H.; Szirmai, M.; Halldin, M. M. Synthesis of Optically Active (−)-11-Nor-delta-9-tetrahydrocannabinol-9-carboxylic Acid. Presented at an International Conference on Cannabis and Cannabinoids, Kolympari, Crete, July 8, 1990; Abstract 5-4.
(21) Calhoun, W.; Chang, J.; Carlson, R. P. Effect of Selected Antiinflammatory Agents and Other Drugs on Zymosan, Arachidonic Acid, PAF and Carrageenan Induced Paw Edema in the Mouse. *Agents Actions* **1987**, *21*, 306–309.

Table IV. Antinociceptive Effects[a]

dose (mg/kg)	2a	1a	6b	3e	6a	3a
0.025	–	–	–	–	10.3 (5)	20.8 (5)
0.050	–	–	–	–	61.7 (5)*	85.0 (5)*
0.10	–	–	–	–	49.5 (20)*	68.3 (5)***
0.25	30.0 (5)*	44.4 (5)*	10.4 (5)	–	61.5 (17)***	33.4 (5)*
0.50	72.5 (5)***	58.5 (5)**	49.0 (10)*	-2.8 (8)	51.7 (8)*	34.0 (5)
1.0	-10.2 (5)	106.1 (5)*	61.4 (15)*	–	14.7 (5)	28.4 (5)
2.0	–	–	37.5 (10)	42.9 (9)	–	–
4.0	–	–	3.1 (10)	–	–	–

[a] Values are the percent change in latency. See the Experimental Section for details. Figures in parentheses are the number of mice, *P < 0.05; **P < 0.01; ***P < 0.005 by a paired t test; otherwise not statistically significant. Under the same conditions indomethacin (10 mg/kg) gave a 51.1% increase in latency and naproxen (40 mg/kg) produced a 64.4% increase (see ref 7 for details).

clinical efficacy. The enantiomeric cannabinoid acids, **3a** and **6a**, were both effective in reducing paw edema in this model as seen in Table I. **3a**, which possesses the same stereochemistry (3*R*,4*R*) as the natural cannabinoids, seems to be more active than its enantiomer, **6a**. Figure 1 shows the time course for inhibition by **3a** at a dose of 0.05 mg/kg given orally. The times shown are the intervals between drug treatment and injection of 1.0 mg of arachidonic acid into the paw. A peak effect was seen at 90 min; however, some protection remained even after 3 h.

A second model, in which edema is induced by platelet activating factor (PAF),[21] was used to evaluate antiinflammatory activity (Table II). Three enantiomeric pairs were tested, and in each case the natural configuration showed greater activity. We also tested the methyl esters, **3e** and **6b**, and the hydroxymethyl precursors, **1a** and **2a**. Interestingly, even though each of these compounds should be metabolized to the corresponding acids in vivo, they showed substantially less activity. Data on the natural pentyl side chain acid is included in Table II that highlights the effect of the modified side chain. The greater than 100% inhibition observed with **3a** was unexpected and, perhaps, reflects the high antiedema potency of this molecule. The biological basis for this effect is not apparent; however, it seems that **3a** can reduce paw fluid content which may not have resulted from PAF injection.

Leukocytes are thought to be major contributors to the inflammatory response, and their ability in this regard is reflected by their adhesiveness to a variety of substrates. The data in Table III show that peritoneal leukocytes from mice given cannabinoids orally exhibit decreased adhesion. In particular, **3a** was most effective, which is in agreement with the data from the paw edema studies (Tables I and II). As in the case of PAF-induced paw edema above, Δ^6-THC-7-oic acid is considerably less active than the synthetic analogues. Compounds **1a** and **2a** produced increases in cell adhesion at a dose 0.05 mg/kg and decreases at higher doses. This perhaps reflects eicosanoid mediation of the drug effects where a complex relationship exists between the various eicosanoids and cell adhesion.

As with many NSAID type drugs, these cannabinoids also showed activity in the mouse hot-plate test (55 °C) for antinociception (Table IV). There appears to be less stereoselectivity in this assay than was observed in the antiinflammatory tests described above (Tables I–III). It is not known whether this reflects fundamental differences in mechanism or is due to less precision in the assay. In addition, the "bell-shaped" dose–response relationships seen in the hot-plate test would support the notion that the analgesic effect differs mechanistically from the other responses reported in this paper. This type of dose–response relationship has been previously reported for cannabinoids.[7]

The lack of CNS activity for the cannabinoid acids is seen in the data shown in Table V. This was measured by the so-called "ring test" in which the cataleptic effects of cannabinoids can be quantitated.[22] The acids all produced little or no response when compared with the parent drug.

Table V. Cataleptic Effects in the Mouse[a]

treatment	dose (mg/kg)	response ± SD
vehicle[b]	–	7.7 ± 4.4
1a	0.1	22.9 ± 10.3*
3a	0.1	5.8 ± 3.4
3a	1.0	12.2 ± 6.0
6a	0.25	12.3 ± 10.3
6a	0.5	13.8 ± 7.9
6a	1.0	10.4 ± 10.6
6a	4.0	8.7 ± 5.6
Δ^6-THC-7-oic acid	5.0	10.1 ± 6.8
Δ^6-THC-7-oic acid	0.5	10.0 ± 7.5
Δ^1-THC	40	48.9 ± 16*

[a] The values are expressed as the means of the fraction of time the mice remained immobile ± SD. See the Experimental Section for other details. An asterisk (*) indicates 95% significance by ANOVA; otherwise not statistically significant. [b] Peanut oil (50 μL) given orally.

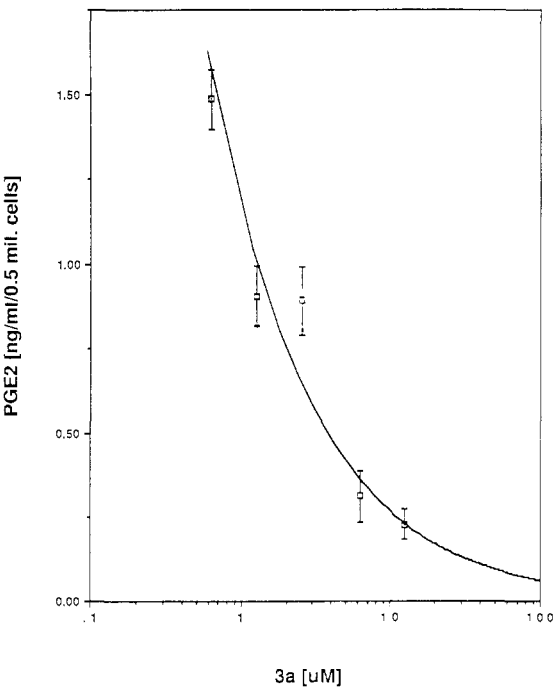

Figure 2. Inhibition of prostaglandin E_2 synthesis by **3a** in mouse peritoneal cells. Cells were prepared as in ref 8 and stimulated by exposure to calcium ionophore (1.0 μg/mL) for 30 min. The media were analyzed for PGE_2 by radioimmunoassay (ref 6) and the values are the means of four replicates ± SE.

(22) Pertwee, R. G. The Ring Test. A Quantitative Method of Assessing the Cataleptic Effect of Cannabis in Mice. *Br. J. Pharmacol.* **1972**, *46*, 753–763.

A possible mechanism that could explain the activities reported here involves inhibition of eicosanoid synthesis by the cannabinoid acids. An example, utilizing an in vitro model, is shown in Figure 2 where PGE_2 production by Ca^{2+}-stimulated mouse peritoneal cells is effectively reduced by prior exposure (30 min) to **3a**. Similar results were observed when leukotriene B_4 (LTB_4) synthesis was monitored in this system (D. Morgan, unpublished results). These findings agree with previous reports[6,8] where we showed that Δ^6-THC-7-oic acid could inhibit both cyclooxygenase and 5-lipoxygenase activities. This suggests that **3a** and **6a** could fall into the category of so-called dual inhibitors.

In summary, the data presented in this report demonstrate that replacement of the *n*-pentyl side chain of Δ^6-THC-7-oic acid with a 1″,1″-dimethylheptyl side chain leads to a large increase in antiinflammatory activity of the cannabinoid acids. This is consistent with analogous studies for other activities where similar increases in potency were observed for such a structural modification.[2-4] Our data also suggest that there is some stereochemical preference for cannabinoid antiinflammatory effects in that the natural 3*R*,4*R* configuration is generally more potent than the 3*S*,4*S* enantiomer. A possible exception to this is the hot-plate test result where the distinction is less clear. The underlying mechanism for the activities reported here is not known; however, it seems reasonable to suggest that effects on eicosanoid production may be involved as was proposed for Δ^6-THC-7-oic acid.[6-8]

Experimental Section

Materials. Arachidonic acid and PAF were purchased from Sigma Chemical Co. (St. Louis, MO). Δ^6-THC-7-oic acid was obtained from Biomol Research, Inc. (Plymouth Meeting, PA) and the Δ^1-THC was supplied by the National Institutes on Drug Abuse (Rockville, MD). The media (MEM) were prepared from Gibco powdered material and penicillin-streptomycin from Sigma was added to control contamination. The culture dishes were Nuclon Delta (sterilized by irradiation).

Chemistry. Melting points were taken in glass capillary tubes with a Thomas-Hoover Uni-Melt apparatus. Infrared spectra were recorded on a JASCO A-200 spectrophotometer. Rotations were determined on a Perkin-Elmer Model 141 polarimeter in chloroform. The microanalyses were performed by the Microanalytical Laboratory of the Hebrew University, and the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Woelm TSC silica, for dry chromatography, activity III/30 mm, No. 04530). The high-resolution mass spectrometry (HRMS) was performed on a Varian 711 instrument at the Mass Spectrometry Center at the Technion, Haifa.

Synthesis of 1a: Dimethyl-*tert*-butylsilyl Ether 4a. This etherification follows the procedure described by Corey and Venkateswarlu.[23] The ester **1b**[13] (2.9 g, 6.17 mmol), $[\alpha]_D$ −152.6° (c 17.2 mg/mL, $CHCl_3$), was dissolved in dry dimethylformamide (DMF) (6 mL). Dimethyl-*tert*-butylsilyl chloride (1.85 g, 12.27 mmol) and imidazole (1.67 g, 24.6 mmol) were added, and the resulting mixture was stirred for 48 h at 38 °C. Water (30 mL) was added, and the mixture was extracted with ether. After evaporation of the dried ether layer an oil (**4b**, 3.6 g) was obtained: $[\alpha]_D$ −153° (c 24.45 mg/mL, $CHCl_3$); IR λ_{max} (neat) 1725 cm^{-1}, no free hydroxyl groups were observed; ^1H NMR ($CDCl_3$) δ 3.28 (1 H, br d, *J* = 16 Hz, C-2 eq H), 4.46 (2 H, s, C-7 H), 5.70 (1 H, m, C-6 H), 6.38 (1 H, d, *J* = 1.5 Hz, arom), 6.42 (1 H, d, *J* = 1.5 Hz, arom). This oil (compound **4b**) was used in the next step with no further purification.

A solution of the above compound (**4b**, 3.2 g, 5.5 mmol) in dry ether (50 mL) was added under a nitrogen atmosphere to lithium aluminum hydride (870 mg) in dry ether (60 mL). The resulting mixture was boiled under reflux for 1.5 h. After the usual workup (ethyl acetate followed by slow addition of a saturated solution of magnesium sulfate until a clear supernatant is formed) the ether layer was dried and evaporated to give an oil (3.2 g). The oil was chromatographed on a silica gel column (100 g), using ether–petroleum ether (6:4) as eluent, to give the alcohol **4a** (8g, 67%): $[\alpha]_D$ −175° (c 7.6 mg/mL, $CHCl_3$); IR λ_{max} (neat) 3320 cm^{-1} (OH band), no carbonyl bands; ^1H NMR ($CDCl_3$) δ 3.38 (1 H, br d, *J* = 16 Hz, C-2 eq H), 4.02 (2 H, s, C-7 H), 5.72 (1 H, br d, C-6 H), 6.36, 6.42 (2 H, s, arom).

Synthesis of Aldehyde 5b. This oxidation follows the procedure described by Corey and Samuelsson.[24] Dry pyridine (2.3 mL) followed by chromic oxide (1.44 g, 14.4 mmol) was added to a solution of methylene chloride–DMF (4:1) (36 mL). The mixture was stirred for 15 min. The primary allylic hydroxy compound **4a** (1.8 g, 3.6 mmol) in methylene chloride–DMF (4:1) (7.2 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. Ethanol (1.8 mL) was added, and the mixture was stirred for an additional 10 min and was then diluted with ethyl acetate (180 mL). The resulting mixture was filtered through a sintered-glass funnel, packed with silica (3 cm), with a layer of anhydrous sodium sulfate on top, and eluted with ethyl acetate (ca 600 mL). The ethyl acetate filtrate was washed with dilute hydrochloric acid (1 N) and then with sodium bicarbonate solution and water. After evaporation of the dried organic solvent a semisolid compound (**5b**, 1.7 g, 95%) was obtained. Crystallization from pentane gave the aldehyde **5b**: mp 80–81 °C; $[\alpha]_D$ −268° (c 6.82 mg/mL, $CHCl_3$); IR λ_{max} 1690 cm^{-1} (neat); ^1H NMR ($CDCl_3$) δ 3.82 (1 H, br d, *J* = 15 Hz, C-2 eq H), 6.38 and 6.42 (2 H, s, arom), 6.80 (1 H, m, C-6 H), 9.50 (1 H, s, C-7 H). Anal. ($C_{31}H_{50}O_3Si$) C, H.

Synthesis of (3*R*,4*R*)-Δ⁶-THC-DMH-7-oic Acid (3a). This oxidation follows the procedure described by Pellegata et al.[14] Sodium chlorite (488 mg) was added portionwise with vigorous stirring to a mixture of the aldehyde **5b** (498 mg, 1 mmol), 2-methyl-2-butene (2.24 mL), saturated aqueous potassium dihydrogen phosphate (1.34 mL), and *tert*-butyl alcohol (22 mL). The reaction mixture was stirred at room temperature for 5 h. Water (20 mL) was added, and the mixture was extracted several times with ethyl acetate, dried, and evaporated to give the crude acid which was purified on a silica gel column (10 g, elution with 10% ether–petroleum ether) to give the acid **3b** (460 mg, 89%) as an oil: $[\alpha]_D$ −218° (c 13.7 mg/mL, $CHCl_3$); IR λ_{max} 1680 cm^{-1} and a broad band in the 2800–3600 cm^{-1} region; ^1H NMR δ 3.75 (1 H, br d, *J* = 18 Hz, C-2 eq H), 6.23 (1 H, d, *J* = 1.5 Hz, arom), 6.27 (1 H, d, *J* = 1.5 Hz, arom), 7.00 (1 H, br d, C-6 H).

Tetrabutylammonium fluoride (0.6 mmol from a 1.0 M solution in THF, Aldrich) was added by injection under a nitrogen atmosphere to a cold solution (ice bath) of the acid **3b** (280 mg, 0.54 mmol) in tetrahydrofuran (THF) (3 mL). The resulting solution was stirred at 0 °C for 15 min. Water was added, and the mixture was extracted several times with ether. The ether layer was dried and evaporated to give the crude product. The product was further purified by silica gel column with ether–petroleum ether (1:1) as eluent. The solid thus obtained (140 mg, 56%) was crystallized from acetonitrile to give the acid **3a**: mp 112–114 °C (sintering); $[\alpha]_D$ −275° (c 3.8 mg/mL, $CHCl_3$); IR λ_{max} (Nujol) 1680 cm^{-1} and a broad band in the 3100–3600-cm^{-1} region; ^1H NMR δ 3.82 (1 H, br d, *J* = 18 Hz, C-2 eq H), 6.22 (1 H, d, *J* = 18 Hz, C-2 eq H), 6.22 (1 H, d, *J* = 1.5 Hz, arom), 6.38 (1 H, d, *J* = 1.5 Hz, arom), 7.16 (1 H, m, C-6 H); *m/z* 400 (M); HRMS calcd for $C_{25}H_{36}O_4$ 400.2613, found 400.2592.

Synthesis of Aldehyde 5a. Tetrabutylammonium fluoride (0.4 mmol from a 1.0 M solution in THF) was added by injection to a cold solution (ice bath) of the aldehyde **5b** (200 mg, 0.4 mmol) in dry THF (4 mL), under a nitrogen atmosphere. The solution was stirred at 0 °C for 5 min and then at room temperature for 15 min. The solvent was evaporated, and the residue was separated on a silica gel column (10 g). The product was eluted with ether–petroleum ether (15:85). The solid obtained (120 mg, 78%)

(23) Corey, E. J.; Venkateswarlu, A. Protection of Hydroxyl Groups as Tert-Butyldimethylsilyl Derivatives. *J. Am. Chem. Soc.* 1972, *94*, 6190.

(24) Corey, E. J.; Samuelsson, B. One Step Conversion of Primary Alcohols in the Carbohydrate Series to the Correponding Carboxylic Tert-Butyl Esters. *J. Org. Chem.* 1984, *49*, 4735.

was crystallized from pentane to give the required compound **5a**: mp 174–175 °C; IR λ_{max} (KBr) 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.84 (1 H, d, J = 17 Hz, C-2 eq H), 6.24, 6.36 (2 Hs, arom), 6.82 (1 H, m, C-6 H), 9.48 (1 H, s, C-7 H). Anal. ($C_{25}H_{36}O_3$) C, H. The methyl ether **5c** melts at 109–110 °C $[\alpha]_D$ –302° (c 8.2 mg/mL, $CHCl_3$); IR λ_{max} (neat) 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.76 (1 H, d, J = 18 Hz, C-2 eq H), 3.80 (3 H, s, OCH_3), 6.38 (1 H, d, J = 1.5 Hz, arom), 6.42 (1 H, d, J = 1.5 Hz, arom), 6.82 (1 H, m, C-6 H), 9.50 (1 H, s, C-7 H); MS m/z 398 (M^+). Anal. ($C_{26}H_{38}O_3$) C, H.

Synthesis of (3*R*,4*R*)-Δ^6-THC-DMH-7-oic Acid Acetate (3c). A solution of the acid **3a** (100 mg, 0.25 mmol) in pyridine (2 mL) and acetic anhydride (1 mL) was stirred overnight at room temperature. Water (5 mL) was added in order to hydrolize any mixed anhydride formed. The mixture was stirred for 2 h and then partitioned between water and ether. The ether layer was washed with dilute HCl (to remove the pyridine) and water. The organic layer was dried and evaporated. Pure product was obtained by preparative TLC (eluent ether–petroleum ether, 60:40) and crystallization from pentane. The acetate **3c**, 65 mg, melts at 120–122 °C: $[\alpha]_D$ –265° (c 9.0 mg/mL, $CHCl_3$); IR λ_{max} (Nujol) 1760 cm^{-1} and a broad band in the 3100–3600-cm^{-1} region; ^{1}H NMR ($CDCl_3$) δ 2.30 (3 H, s, $OCOCH_3$), 3.38 (1 H, br d, J = 19 Hz, C-2 eq H), 6.56 (1 H, d, J = 1.5 Hz, arom), 6.68 (1 H, d, J = 1.5 Hz, arom), 7.18 (1 H, m, C-6 H); HRMS calcd for $C_{27}H_{38}O_5$ 442.2719, found 442.2691. Anal. ($C_{27}H_{38}O_5$) C, H.

Synthesis of (3*R*,4*R*)-Δ^6-THC-DMH-7-oic Acid, Methyl Ether (3d). Compound **1a** (1 g) was dissolved in DMF (10 mL). Potassium carbonate (1.96 g) and methyl iodide (3 mL) were added, and the mixture was stirred at room temperature for 4 h. Water (40 mL) and ether (20 mL) were added, and the organic layer was dried and evaporated. The oily material **4c** was oxidized without further purification, following the above described procedure for the synthesis of **5b**, using dry pyridine (1.5 mL), methylene chloride (20 mL), DMF (5 mL), and chromic oxide (1 g). The above-obtained cannabinoid ether **4c** was added in solution of methylene chloride (4 mL) and DMF (1 mL). The workup included addition of ethanol (1.25 mL) and ethyl acetate (125 mL) as described above. The isolated reaction product was crystallized from pentane to give the methyl ether **5c**: 0.77 mg; mp 109–110 °C; $[\alpha]_D$ –302° (c 8.2 mg/mL, $CHCl_3$); IR λ_{max} (neat) 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.76 (1 H, d, J = 18 Hz, C-2 eq H), 3.80 (3 H, s, OCH_3), 6.38 (1 H, d, J = 1.5 Hz, arom), 6.42 (1 H, d, J = 1.5 Hz, arom), 6.82 (1 H, d, J = 1.8 Hz, C-6 H), 9.50 (1 H, s, CHO); MS m/z 398 (M^+). Anal. ($C_{26}H_{38}O_3$) C, H.

The aldehyde **5c** was oxidized to the acid **3d** following the procedure described for the acid **3b**. Sodium chlorite (488 mg) was added portionwise with vigorous stirring to a mixture of the aldehyde **5c** (398 mg, 1 mmol), 2-methyl-2-butene (2.24 mL), saturated aqueous potassium dihydrogen phosphate (1.34 mL), and *tert*-butyl alcohol (22 mL). The reaction was stirred at room temperature for 5 h. Water (20 mL) was added, and the mixture was extracted several times with ethyl acetate, dried, and evaporated to give the crude acid which was purified on a silica gel column (10 g, elution with 10% ether–petroleum ether) to give the acid, methyl ether **3d**: mp 172–174 °C; $[\alpha]$ –270° (7.5 mg/mL, $CHCl_3$); IR λ_{max} (KBr) 1660, 1680 cm^{-1} and a broad band in the 3100–3600-cm^{-1} region; ($CHCl_3$) 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.76 (1 H, br d, J = 18 Hz, C-2 eq H), 3.80 (3 H, s, OCH_3), 6.39, 6.42 (2 H, s, arom), 7.16 (1 H, m, C-6 H); MS m/z 414 (M^+). Anal. ($C_{26}H_{38}O_4$) C, H.

Synthesis of (3*R*,4*R*)-Δ^6-THC-DMH-7-oic Acid, Methyl Ester (3e). The acid **3b** (30 mg) was dissolved in ether (5 mL). A deep yellow solution of diazomethane (5 mL) (prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, 21 g, in ether, 250 mL, by reaction with potassium hydroxide, 5 g, in 8 mL of water) was added, and the yellow solution was left at room temperature for 1 h. On TLC one spot, different from the starting material, was noticed. The solution was evaporated, and the resulting oil (30 mg) was dissolved in dry THF (2 mL). The protecting group was removed as described above with tetrabutylammonium fluoride to give an oil (30 mg) which was purified on preparative TLC. The methyl ester, **3e** (20 mg), thus obtained gave one spot on TLC and one peak on GC which comprised 95% of the area of the peaks appearing after the solvents area. **3e**: oil $[\alpha]_D$ –211° (c 12 mg/mL, $CHCl_3$); IR λ_{max} (neat) 3400 (OH), 1715, and 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (3 H, s, $COOCH_3$), 6.25 (1 H, d, J = 1.5 Hz, arom), 6.36 (1 H, d, J = 1.5, arom), 7.01 (1 H, d, J = 2.6 Hz, C-6 H); MS m/z 414 (M^+). Compound **3e** can also be obtained from the free acid **3a**, by direct esterification with diazomethane following the procedure described above for **3b**.

Upon base catalized hydrolysis **3e** gave the crystalline acid **3a**: **3e** (100 mg) was dissolved in methanol (30 mL), a sodium hydroxide solution (7 mL, 20% aq) was added, and the mixture was refluxed for 3 h. The methanol was evaporated, and ether (20 mL) and hydrochloric acid (1 N) were added until the aqueous phase became acidic. The organic solution was washed with water, dried, and evaporated. The solid residue was crystallized from ether–pentane to give the acid **3a**, 40 mg, mp 108–111 °C, identical by IR to **3a** prepared as described above.

Syntheses of 3*S*,4*S* Enantiomers. These preparations followed exactly the syntheses described above for the 3*R*,4*R* series. The physical data of the 3*S*,4*S* compounds are identical to those of the corresponding compounds in the 3*R*,4*R* series, except for the rotation which has the same absolute value but opposite direction. The acid **6a**, which was the investigated compound in 3*S*,4*S* series, has a mp 112–114 °C (from acetonitrile) (sintering): $[\alpha]_D$ 278° (c 4.1 mg/mL, $CHCl_3$); IR λ_{max} (Nujol) 1680 cm^{-1} and a broad band in the 3100–3600-cm^{-1} region; ^{1}H NMR δ 3.82 (1 H, bd, J = 18 Hz, C-2 eq H), 6.22 (1 H, d, J = 1.5 Hz, arom), 6.38 (1 H, d, J = 1.5 Hz, arom), 7.16 (1 H, m, C-6 H); HRMS calcd for $C_{25}H_{36}O_4$ 400.2613; found 400.2628.

Pharmacology. PAF- and Arachidonic Acid-Induced Paw Edema. The conditions were based on those reported previously.[8,21] Water was substituted for mercury as the displacement medium and was found to give satisfactory results. PAF (1.0 μg) or arachidonic acid (1.0 mg) dissolved in 50 μL of 5% ethanol in saline was injected sc into the plantar surface of the right hind paw of CD-1 female mice (20–25 g) obtained from Charles River Labs. The mice were under ether anesthesia during this procedure. The volume of the right foot was measured to the level of the lateral malleous by water displacement before treatment and 15 min after PAF injection or 30 min after arachidonate injection. The change in paw volume was calculated for each mouse and the significance for each group was determined by a paired t test analysis.

Leukocyte Adhesion Assay. The details of this assay have been previously reported by us.[25] In brief, peritoneal cells from female CD-1 mice (20–25 g) were collected at 90 min following oral administration of the drug or vehicle (50 μL of peanut oil). Cells from each treatment group (N = 3) were pooled, and equal numbers were aliquoted into six culture dish wells (1.9-cm^2 area). After incubation for 18–20 h, nonadhering cells were removed and the remaining cell monolayer quantitated by DNA measurement. Cell viability was monitored by Trypan Blue exclusion.

Hot-Plate Test for Antinociception. The hot-plate test for analgesia was adapted from the procedure described by Kitchen and Green.[26] An aluminum surface was maintained at 55 ± 1 °C by circulating water through passages in the metal. A clear plastic cylinder 18 cm in diameter and 26 cm high was placed on the surface to prevent escape. The end point was taken when the mouse either performed a hind paw lick or jumped off the surface; in no case were the animals kept more than 30 s on the plate. Mice were never used more than one time; control values were always measured at 11 a.m. and test values at 2 p.m. The drugs were administered orally 90 min before the hot plate test. The percent change in response time (latency) was calculated by comparing the mean of the control values with the mean of the test values and statistical significance determined by a paired t test analysis using software (Statview, 512) from Brainpower Inc., Calabasas, CA.

Measurement of Cataleptic Effects. The cataleptic response was measured using the ring test described by Pertwee.[22] Mice

(25) Audette, C. A.; Burstein, S. Inhibition of Leukocyte Adhesion by the *In Vivo* and *In Vitro* Administration of Cannabinoids. *Life Sci.* **1983**, *47*, 753–759.
(26) Kitchen, I.; Green, P. G. Differential Effects of DFP Poisoning and Its Treatment on Opioid Antinociception in the Mouse. *Life Sci.* **1983**, *33*, 669–672.
(27) Burstein, S. Platelet Activating Factor Antagonist and Methods of Use Therefor. U.S. Patent 4,973,603, 1990.

were placed on a horizontal wire ring 5.5 cm in diameter, which was attached to a 16-cm vertical rod. The hind paws and fore paws were placed at opposite sides of the ring. It is important that the ambient temperature is maintained at 30 °C and that the environment be free of auditory stimuli and bright lights. The criteria for immobility are detailed in ref 22. The response is calculated as the fraction of time the mouse is immobile over a 5-min test period. Measurements were always done between 2 and 4 p.m. and the animals were used only once.

Acknowledgment. We thank NIDA for partial support (Grants DA02052, DA06481 and a Research Scientist Award to SHB) and Prof. M. A. Tius, University of Hawaii, for helpful correspondence.

Registry No. **1b**, 113418-02-3; **3a**, 137945-48-3; **3b**, 137945-49-4; **3c**, 137945-50-7; **3d**, 137945-51-8; **3e**, 137945-52-9; **4a**, 137945-53-0; **4b**, 137945-54-1; **4c**, 137945-55-2; **5a**, 137945-56-3; **5b**, 137945-57-4; **5c**, 137945-58-5; **6a**, 137945-59-6; **6b**, 137945-60-9.

Synthesis and Cholinergic Properties of *N*-Aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamino Analogs of Ranitidine

Matthew J. Valli,† Yunzhao Tang,‡ J. W. Kosh,§ James M. Chapman, Jr.,*,§ and J. Walter Sowell, Sr.§

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Columbia, South Carolina 29208, and Department of Pharmacology, Shanxi Medical College, Taiyuan, Shanxi 030001, People's Republic of China. *Received January 30, 1992*

A series of *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamino analogs of the H_2-antagonist, ranitidine, was synthesized and the abilities of the compounds to alleviate the cholinergic deficit characteristic of Alzheimer's disease evaluated. The compounds were initially tested for their ability to inhibit human erythrocyte acetylcholinesterase activity in vitro. Selected compounds were further evaluated for butyrylcholinesterase inhibition, M_1 and M_2 cholinergic receptor binding, potentiation of ileal contractions, and the ability to elevate brain acetylcholine levels in mice. The analogs were compared to tetrahydroaminoacridine and to a recently reported series of bis[[(dimethylamino)methyl]furans]. The *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine derivatives were generally comparable to tetrahydroaminoacridine and the bis[[(dimethylamino)methyl]furans] in acetylcholinesterase inhibition, M_1/M_2 receptor binding, and the potentiation of ileal contractions, while being more potent inhibitors of acetylcholinesterase than butyrylcholinesterase. The 4-nitro-3-pyridazinyl analog, **26**, was notable in demonstrating a potent and selective binding to the M_2 receptor, with an M_2 IC_{50}/M_1 IC_{50} of 0.060. Compounds in which the substituents on the dinitro-*N*-aryl moiety were relatively small were the best at inhibiting acetylcholinesterase in vitro. The *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamines in general, and those with small *N*-aryl substituents in particular, were superior to the bis[[(dimethylamino)methyl]furans] in elevating brain ACh levels in mice, probably due to enhanced distribution into the CNS. The 1,5-difluoro-2,4-dinitrophenyl analog, **8**, resulted in the largest elevation in brain acetylcholine levels, affording a 53% increase at 88 mg/kg.

In a previous communication, we described the synthesis and cholinergic properties of a series of bis[[(dimethylamino)methyl]furan] analogs of ranitidine.[1] These compounds possessed the general structure shown in Figure 1, and demonstrated potent acetylcholinesterase (AChE) inhibitory activity in vitro for a wide variety of substituents, "Z". Compound 1 (IC_{50} = 0.03 μM) was the most potent AChE inhibitor in the series and was found to be approximately 6 times more potent than tetrahydro-9-aminoacridine (THA), which is currently undergoing extensive clinical investigation in the treatment of Alzheimer's disease (AD). A number of these analogs also exhibited an enhanced selectivity for AChE inhibition vs butyrylcholinesterase (BChE) inhibition, possessed M_1/M_2 muscarinic receptor affinities similar to THA, and potentiated acetylcholine-induced contractions of isolated rat ileum. The bis[[(dimethylamino)methyl]furans], however, showed little ability to elevate mouse brain acetylcholine levels in vivo, with the most potent compound, **2**, demonstrating a 22% increase at 80% of its approximate lethal dose. The relatively high molecular weights of these compounds and the presence of two very basic tertiary, aliphatic amino groups probably limited distribution into the central nervous system (CNS).[1]

Utilizing compound 1 as a prototype, we have therefore synthesized a series of *N*-aryl derivatives of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine with the general structure shown in Figure 2. We have also synthesized N-substituted analogs of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine, containing 2-cyano-3-fluorophenyl, 2-nitro-4-fluorophenyl and 4-nitro-3-pyridazinyl moieties.

In comparison to compound 1, the series of compounds in the present work had lower molecular weights and generally possessed a single, tertiary, aliphatic amino group. It was postulated that these changes would augment distribution across the blood–brain barrier, thus allowing these compounds to display greater cholinergic effects within the CNS.

Chemistry

N-Arylation of the primary amine[2] **3** with either 2,4-dinitrofluorobenzene, 1,5-difluoro-2,4-dinitrobenzene, 2,6-difluorobenzonitrile, or 2,5-difluoronitrobenzene in acetonitrile in the presence of anhydrous sodium carbonate yielded compounds **4–7**, respectively. These products were

† Current address: Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.
‡ Shanxi Medical College.
§ University of South Carolina.

(1) Sowell, J. W., Sr.; Tang, Yunzhao; Valli, M. J.; Chapman, J. M., Jr.; Usher, L. A.; Vaughn, C. M.; Kosh, J. W. Synthesis and cholinergic properties of bis[[(dimethylamino)methyl]furanyl] analogs of ranitidine. *J. Med. Chem.* **1992**, *35*, 1102–1108.
(2) Price, B. J.; Clitherow, J. W.; Bradshaw, J. Patentschrift (Switz.) 647, 517. Furanalkanamide derivatives. *Chem. Abstr.* 1986, *104*, P 168352f.

0022-2623/92/1835-3141$03.00/0