Table I

Compound	Yield, %	Mp, °C[a]	Formula[a]
N,N-Dimethyl-*p*-(1-dibenzofuryl)aniline (1)	53	159–161	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(2-dibenzofuryl)aniline (2)	60	214–215	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(3-dibenzofuryl)aniline (3)	60	256–258	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(4-dibenzofuryl)aniline (4)	90	160–161	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(5-benzofuryl)aniline (5)	72	136–137	$C_{16}H_{15}N_3O$
N,N-Dimethyl-*p*-(6-benzofuryl)aniline (6)	85	127–128	$C_{16}H_{15}N_3O$
N,N-Dimethyl-*p*-(7-benzofuryl)aniline (7)	69	134–136	$C_{16}H_{15}N_3O$

[a] All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Where analyses are indicated only by the symbols of the elements, analytical results obtained for carbon and hydrogen were within ±0.4% of the theoretical values.

Experimental Section

1-Aminodibenzofuran. 4-Aminodibenzofuran was synthesized according to Gilman[8] in 50% yield and acetylated in 80% yield. Bromination gave a quantitative yield of the 1-bromo compound which was hydrolyzed and converted to 1-bromodibenzofuran.[8] This material (7 g) was heated to 210° for 24 hr with cuprous bromide and ammonium hydroxide to give 6.5 g of the 1-aminodibenzofuran hydrochloride after treatment of the ether extraction of the autoclave reaction product with dry HCl. The free amine melted at 74°.[8]

2-Aminodibenzofuran. This compound was prepared from 2-iododibenzofuran by heating with ammonium hydroxide as above. The yield was 96% melting at 127°.[14]

3-Aminodibenzofuran. This compound was prepared by catalytic hydrogenation of 3-nitrodibenzofuran.

4-Aminodibenzofuran. This material was prepared in 50% yield, melting at 85°.[10]

5-Aminobenzofuran. Reduction of 5-nitrobenzofuran[15] with 40 psi of hydrogen using 10% Pd/C catalyst gave a quantitative yield of this amine as the hydrochloride melting at 253–255°.

6-Aminobenzofuran. 6-Nitrodibenzofuran[16] was reduced in the above manner and obtained as the hydrochloride salt.[17]

7-Aminobenzofuran. This amine was obtained as the hydrochloride melting at 240–250° by hydrogenation in the above manner of 7-nitrobenzofuran.[15]

N,N-Dimethyl-*p*-(*x*-dibenzofuryl)anilines. A typical procedure for all of these isomers is given. 4-Aminodibenzofuran (18.3 g, 0.10 mol) as the hydrochloride (21.9 g, 0.10 mol) was dissolved in warm water (200 ml). Concentrated hydrochloric acid (17 ml, 0.20 mol) was added and the mixture was quickly cooled by ice addition. Sodium nitrite (7.6 g, 0.11 mol) dissolved in water (50 ml) was added quickly with good stirring, and the stirring was continued for 30 min. Sulfamic acid was then added until a negative test with starch-iodide paper was observed. Sodium acetate (as a powder) was added to adjust the pH to 6–8. The mixture was filtered and transferred to a beaker, and *N,N*-dimethylaniline (14 ml, 0.11 mol) in ethanol (50 ml) was added in a steady stream with good stirring. Coupling occurred immediately and, after allowing the reaction to continue for 30 min, the solid was removed by filtration and water washed. The dried filter cake was dissolved in benzene (hot) and on cooling and filtering the 4-(*p*-*N,N*-dimethylaminophenylazo)dibenzofuran was obtained in 90% yield (29 g) (see Table I).

N,N-Dimethyl-*p*-(*x*-dibenzofuryl)anilines. These azo compounds were made in a similar manner to the dibenzofuran products above and are reported in Table I.

Biological Properties. Young male rats of the Sprague–Dawley strain, approximately 8 weeks of age and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of ten animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller, *et al.*,[12] to which had been added one of the azo compounds at a level of 0.03%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; cerelose, 770 g; Osborne and Mendel salt mixture, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate 67,500 IU.

In each experiment, groups received DAB at the 0.06 as well as at the 0.03% level. The control group received only the basal diet. All of the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Typical Pathologist's Reports. 5. Regenerating nodules of hepatic parenchymal cells entrapped by wide bands of proliferating and invasive bile duct and cholangiolar elements, the latter showing marked focal anaplasia. Diagnosis: bile duct carcinoma.

7. Marked proliferation of cholangiolar and bile duct epithelium, widely invading the hepatic parenchyma and displacing hepatic cells. Tumor forms duct-like and glandular structures. Diagnosis: bile duct carcinoma.

Results and Discussion

In the biological evaluation DAB (Butter yellow) at the 0.06% level gave tumor incidences of 7/10 at 4 months and 9/10 at 6 months, while at the 0.03% level it gave 5/10 in 6 months. Our most active compound, **7**, at the 0.03% level gave 10/10 tumors in 3 months. **5** gave 10/10 tumors at 4 months at the 0.06% level and **6** gave no tumors at 6 months at the 0.06% level. The order of their carcinogenicity is **7** >> **5** >> **6** and all the dibenzofuran analogs were inactive at 8 months and the 0.06% level.

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Stereoselectivity of Cholinergic Activity in a Series of 1,3-Dioxolanes

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We have previously described[1] the utility of the 1,3-dioxolane structure as a basic nucleus of potent agonists and antagonists active at the muscarinic acetylcholine receptors of smooth muscle. 2-Methyl-4-dimethylaminomethyl-1,3-

Table I. Physical Data for 2,2-Dialkyl-4-dimethylaminomethyl-1,3-dioxolane Methiodides

2,2-Dialkyl substituent		Mp, °C[a]	$[\alpha]^{25}D$[b]	Analyses
Me_2	*R*	218–220	+11.4	C, H, I ($C_9H_{20}INO_2$)
Me_2	*S*	219–221	−11.2	C, H, I ($C_9H_{20}INO_2$)
Et_2	*R*	159–161	+7.89	C, H, I ($C_{11}H_{24}INO_2$)
Et_2	*S*	159–160	−7.75	C, H, I ($C_{11}H_{24}INO_2$)
i-Pr_2	*R*	211–212	+3.51	C, H, I ($C_{13}H_{28}INO_2$)
i-Pr_2	*S*	212	−3.42	C, H, I ($C_{13}H_{28}INO_2$)

[a] Recrystallization solvent, PrOH. [b] *c* 10, MeOH.

dioxolane methiodide (CD) is a highly selective and active agonist[2] with substantial stereoselectivity of action; the cis isomer is some 5–10 times more active than the trans isomer[2] and the 2*S*,4*R* (L-cis) is some 100 times more active than the 2*S*,4*S* (D cis) isomer.[3,4] Recent investigations on anticholinergic 1,3-dioxolanes[1,5] carrying nonpolar functions at the 2 position have revealed, however, an almost complete lack of stereoselectivity of antagonistic activity. We felt it to be of interest, therefore, to examine a series of 1,3-dioxolanes bearing 2 substituents that would generate a transition in activities between agonists and antagonists. Accordingly, we have synthesized and examined the 2,2-dimethyl- (DMD), 2,2-diethyl- (DED), and 2,2-diisopropyl- (DPD) 4-dimethylaminomethyl-1,3-dioxolane methiodides as both *R* and *S* (4 position) isomers. To extend the comparison we have also included data for (*R*)- and (*S*)-acetyl-β-methylcholine.

Experimental Section

Melting points were recorded on a Thomas-Kofler hot stage and are corrected. Elemental analyses were performed by Dr. A. E. Bernhard and were within 0.4% of the theoretical values.

Preparation of Compounds. The starting materials for the dioxolanes were (*R*)- and (*S*)-1-tosyloxyglycerol.[3] The *R* and *S* enantiomers of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide were prepared by the method of Belleau and Puranen.[3] The 2,2-dimethyl-, 2,2-diethyl-, and 2,2-disopropyl-4-dimethylaminomethyl-1,3-dioxolane methiodides were prepared from the 1-tosyloxyglycerols and the appropriate ketone in refluxing PhH with azeotropic removal of water (*p*-toluenesulfonic acid was used as catalyst).[1] Physical constants are summarized in Table I; the intermediate 2,2-dialkyl-4-tosyloxymethyl-1,3-dioxolanes were not characterized.

Pharmacological Activities. Activities were determined using longitudinal muscle from the guinea-pig ileum as previously reported.[1,6] The pD_2 values are the negative logarithms of the ED_{50} values and were unchanged by hexamethonium (100 mg/1000 ml). π (hydrophobicity) values for the compounds were calculated from the substituent constant compilation of Fujita, *et al.*,[7] using an arbitrary value of π = 0 for the parent 4-dimethylaminomethyl-1,3-dioxolane methiodide nucleus common to all of the compounds studied.

Results and Discussion

Table II summarizes the experimental findings. These data confirm the well-documented high stereoselectivity of acetyl-β-methylcholine interaction at the muscarinic receptor and the identical chirality of interaction of CD[3,4] although the latter shows reduced stereoselectivity relative to acetyl-β-methylcholine. Progressive substitution of 2-alkyl groups in the dioxolanes produces the anticipated decrease of affinity as agonists and this is accompanied by a reduction in stereoselectivity until with DPD there is an actual inversion in the stereoselectivity (*R*/*S*) ratio.

From the data of Table I it is apparent, however, that the 2 substituents are actually making different contributions to the parameters intrinsic activity (i.a.) and apparent affinity (pD_2). In the *R* series progressive alkyl substitution causes a decrease in pD_2 values but does not affect i.a. which remains at 1.0 (Figure 1). The parent (2*S*,4*R*)-*cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide deviates very significantly from this relationship (Figure 1) and again indicates the previously emphasized[1,9] demanding structural requirements for high agonist activity in this receptor system. Extrapolation of this equation to π = 0 gives a pD_2

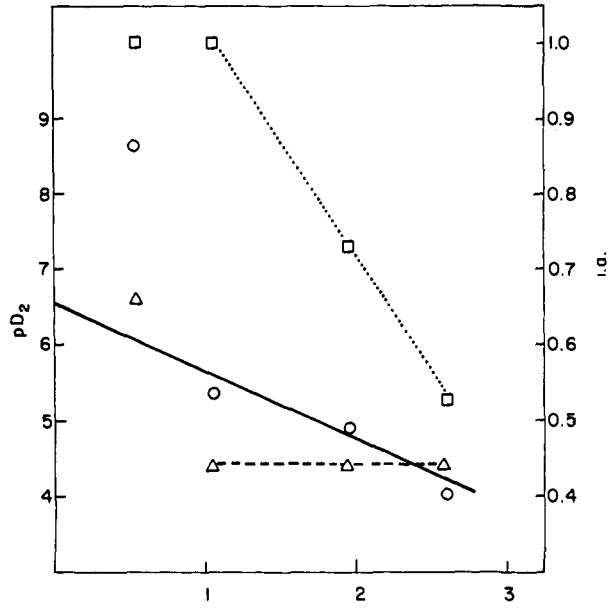
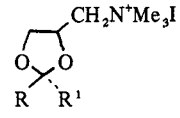


Figure 1. Plot of pD_2 and i.a. against π for 1,3-dioxolanes listed in Table II: △, pD_2 values of *S* enantiomers; ○, pD_2 values of *R* enantiomers; □, i.a. values of *S* enantiomers.

Table II. Muscarinic Activities of Stereoisomers of 1,3-Dioxolanes and Acetyl-β-methylcholine in Guinea-Pig Ileal Longitudinal Smooth Muscle



R	R'	Isomer	ED_{50} ± S.E.M.	pD_2[a]	i.a.[b]	*R*/*S*[c]	δ	*n*[d]
CH_3	H	*R* (L)	$2.3 \pm 0.2 \times 10^{-9}$	8.64	1	104	0.52	16
(CD)		*S* (D)	$2.4 \pm 0.1 \times 10^{-7}$	6.62	1			8
CH_3	CH_3	*R* (L)	$4.3 \pm 0.1 \times 10^{-6}$	5.37	1	8.4	1.04	8
(DMD)		*S* (D)	$3.6 \pm 0.2 \times 10^{-5}$	4.44	1			8
C_2H_5	C_2H_5	*R* (L)	$1.2 \pm 0.1 \times 10^{-5}$	4.92	1	3.2	1.94	8
(DED)		*S* (D)	$3.8 \pm 0.4 \times 10^{-5}$	4.42	0.73 ± 0.04			8
$CH(CH_3)_2$	$CH(CH_3)_2$	*R* (L)	$9.3 \pm 0.5 \times 10^{-5}$	4.03	1	0.4	2.60	8
(DPD)		*S* (D)	$3.8 \pm 0.5 \times 10^{-5}$	4.42	0.53 ± 0.08			8
Acetyl-β-methylcholine		*S* (L)	$5.8 \pm 0.5 \times 10^{-8}$	7.24	1	1000 (*S*/*R*)		12
		R (D)	$6.0 \pm 0.8 \times 10^{-5}$	4.22	1			12

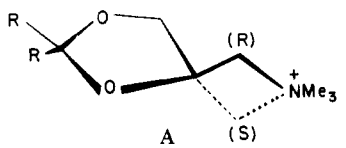
[a] $pD_2 = -\log ED_{50}$. [b] i.a. is a measure of the maximum response produced by an agonist relative to *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide. [c] *R*/*S* is the ratio of ED_{50} of the stereoisomers in question. [d] *n* is the number of preparations.

value of 6.33 for (*R*)-4-dimethylaminomethyl-1,3-dioxolane methiodide, approximately that reported by Belleau and Lavoie.[9]

In marked contrast, in the *S* series progressive alkyl substitution causes an initial decrease in pD_2 value with DMD and this is approximately equal in both the *R* and *S* series. Additional alkyl substitution produces no further change in pD_2 but rather causes a progressive decrease in i.a. (Figure 1).

Two points deserve emphasis from these findings. First, whether one considers this series of dioxolanes as the racemates[1] or as the enantiomers, the parent compound CD has uniquely high agonist activity. Obviously, this must reflect a contribution made by the 2-methyl group of CD beyond that predicted by the π value; quite possibly this indicates a highly productive interaction of this ligand with the receptor relative to other members of this series.[1,9] The second point of emphasis concerns the divergent effects of 2,2-dialkyl substitution upon the pD_2 and i.a. parameters and the ultimate inversion in stereoselectivity. Despite the fact that these parameters are probably complex functions of the total transduction sequence initiated by the agonist-receptor interaction,[10] some tentative conclusions concerning the significance of the data in Table I do seem possible.

Previous work aimed at defining the active conformation of these agents when bound at the muscarinic receptor suggests a conformation (see structure A) in which the $-N^+Me_3$



group is maximally extended from the dioxolane ring.[11,12] Examination of models of the enantiomeric pairs of the 2,2-dialkyl-substituted dioxolanes listed in Table I shows that they can present in identical fashion the same functional groups to a receptive surface save for the methylene group of the trimethylaminomethyl side chain (structure A). It would thus be anticipated that there would be a constant difference in activity (pD_2, i.a., or both) between each enantiomeric pair produced by this single difference of interaction. This is clearly not so and other explanations must be sought.

The explanation that we wish to advance is based on our previously expressed hypothesis[12,13] that there may exist partially overlapping subsites with different molecular requirements (approximately "polar" and "nonpolar," Figure 2) for ligands active at the muscarinic receptor and on the fact that β-Me substitution into ACh serves not to increase muscarinic activity but only to maintain it in the *S* enantiomer and to decrease it in the *R* enantiomer.[12,14] Accordingly, with increasing nonpolar (2-alkyl) substitution in the 1,3-dioxolane series, there is an increasing tendency for binding in the nonpolar area which is more pronounced for the 4-*S* enantiomers because of the detrimental β-Me effect. The activity difference between (*R*)- and (*S*)-CD probably represents a true stereoselectivity of interaction at the polar subsite but further substitution may cause the two enantiomers to bind in different subsites. At the polar subsite the effects of addition 2-alkyl substitution into CD are seen as decreases in affinity of the *R* enantiomers while at the nonpolar subsite the same substitutions have no effect on affinity but decrease i.a. and this results in the observed inversion of stereoselectivity at DPD. It is of interest that it is at hexyl N^+Me_3 in the alkyltrimethylammonium series that i.a. decreases from 1.0 to 0.9 and that the effective

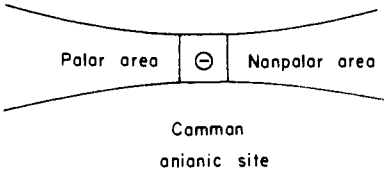


Figure 2. Schematic representation of the muscarinic receptor site showing a common anionic area with adjacent polar and nonpolar subsites for interaction with polar and nonpolar side chains, respectively, of agonist molecules.

chain length in DED ($C_2H_5CO_1CCN^+$) is also C_6.[8,12] This may represent the transition point in binding orientation.

The proposal advanced above is to be recognized as tentative; nevertheless, it accords with previous proposals of multiple ligand binding orientations in this and other macromolecular systems[12] including such enzymes as carboxypeptidase,[15] subtilisin,[16] and, of particular relevance, acetylcholinesterase.[17,18] Furthermore, the work reported here suggests a cautionary note in the interpretation of stereoselectivity data.

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Analgetic-Antiinflammatory Activity of Prenyl Derivatives of Basic Naphthylalkylnitriles and Naphthylalkylamides Chemically Related to Naphthypramide

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In prior communications,[1,2] it has been reported from this laboratory that α-isopropyl-α-(2-dimethylaminoethyl)-1-naphthylacetamide (naphthypramide) possesses a noteworthy