Inhibitors of Hepatic Mixed Function Oxidase. 3. Inhibition of Hepatic Microsomal Aniline Hydroxylase and Aminopyrine Demethylase by 2,6- and 2,4-Dihydroxyphenyl Alkyl Ketones and Related Compounds

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A series of 2,6- and 2,4-dihydroxyphenyl alkyl ketones has been investigated as inhibitors of hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities. Structural alterations in both series did little to enhance the inhibitory activity of the parent compounds 2,6-dihydroxyacetophenone (3) and 2,4-dihydroxyacetophenone (27). In the 2,6 series activity against both microsomal systems varied only over a relatively narrow range, 6-allyloxy-2-hydroxyacetophenone (19) being the most potent inhibitor. In the 2,4 series, activity against aniline hydroxylase was poor or absent in most cases. The most potent inhibitor was 5-ethyl-2,4-dihydroxyacetophenone (31). In contrast, high activity against aminopyrine demethylase was frequently displayed in this series, 3,5-dibromo-2,4-dihydroxypropiophenone (36) showing greatest inhibitory potency. The effects of some compounds on hexobarbital sleeping times and zoxazolamine paralysis times in mice were also examined.

The mixed function oxidase (MFO) system of liver is largely responsible for the oxidative metabolism of xenobiotics to more polar and generally less toxic substances.[1] However, it has also been shown to be important in the activation of carcinogenic polycyclic hydrocarbons and other toxic agents.[2] Inhibition of the MFO system is the basis of the mode of action of insecticidal synergists[3] and it is reasonable to expect that inhibitors of the MFO system may have synergistic properties. A number of compounds have been found to inhibit mammalian MFO including SKF 525-A,[4] piperonyl butoxide and related compounds,[5] 1-substituted imidazoles,[6-8] and benzimidazoles, benzoxazoles, and benzothiazoles.[9,10] Discussions of the structure–activity relationships (SAR) of these inhibitors were presented.[5-10]

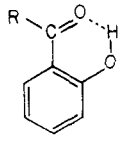
Recently we found that a number of cyclic β-triketones extracted from Australian plants were effective inhibitors of rat liver MFO activity.[11] The common structural feature present in these compounds was the 2,4,6-trioxyphenyl ketone system. The present paper presents the results of SAR studies with a series of 2,6- and 2,4-dihydroxyphenyl alkyl ketones and derivatives prepared in an attempt to design efficient inhibitors of aniline hydroxylase and aminopyrine demethylase of rat hepatic microsomes. These activities are typical mixed function oxidases which involve cytochrome P-450.[1] The enzyme substrates were chosen to be representative of the two principal groups of compounds which afford difference spectra with oxidized cytochrome P-450.[36] Aminopyrine affords a type I difference spectrum while aniline affords a type II.

Results and Discussion

The common structural feature present in the compounds examined was the 2-hydroxyaryl ketone system seen in the simplest structure in 2-hydroxyacetophenone (1). Two series of compounds containing the 2-hydroxyphenyl ketone system were investigated, 2,6-dihydroxyphenyl ketones and derivatives (Table I) and 2,4-dihydroxyphenyl ketones and derivatives (Table II). Most compounds in both series possess a planar six-membered hydrogen-bonded ring (Chart I). Comparison of enhancement of aniline hydroxylase activity by acetophenone[12] and the I_{50}'s of 2-hydroxyacetophenone (1) and 2,6-dimethoxyacetophenone (24) suggests that this hydrogen-bonded ring is necessary for the compound to have inhibitory properties toward aniline hydroxylase. This ring need not be present for inhibitory action toward aminopyrine demethylase since acetophenone,[13] 1, and 24 all display inhibitory properties.

Alteration of Acyl Group. The effect of modifying the acyl group in the 2,6-dihydroxyphenyl ketone series

Chart I

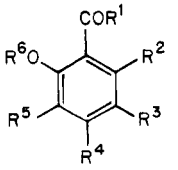


is shown by the inhibitory properties of compounds 2–7 (Table I). Inhibition of aniline hydroxylase activity is maximal with a propionyl group (4) and declines thereafter, apparently with increasing size of the substituent (5–7). Aminopyrine demethylase activity is inhibited in a similar fashion, compound 5, with a butyryl group, being the most inhibitory compound. Compounds in the 2,4-dihydroxyphenyl alkyl ketone series (Table II) are less potent inhibitors than the analogous compounds in the 2,6-disubstituted series. (Compare 2–5 with 26–29). Inhibitory potency toward aminopyrine demethylase appeared greatest with the butyryl side chain in this series of compounds. The butyryl (29) and isobutyryl (30) derivatives were equipotent against both enzyme activities and were more potent than the acetyl (27) and propionyl (28) derivatives. In both series it is noteworthy that the aldehydes 2 and 26 displayed little activity against aminopyrine demethylase but there is an impressive increase in activity in the acetophenones 3 and 27. The data obtained clearly indicate that substantial structural alteration of the acyl group in these compounds does little to enhance inhibitory activity. Overall the dihydroxyphenyl propyl ketones appeared to be the most potent inhibitors.

Etherification of Phenolic Hydroxyl. In the 2,6-dihydroxyacetophenone derivatives (Table I) monoetherification did not enhance activity to any great degree (3, 15–22). 2-Hydroxy-6-methoxyacetophenone (15) was about as potent as its parent (3), and lengthening of the alkoxy group (15–17) or insertion of allyloxy (19) or benzyloxy (20) groups did not improve potency. Interestingly, the *n*-butyl ether (18) showed good potency against aminopyrine demethylase but was the least active of the alkyl ethers as an inhibitor of aniline hydroxylase.

The 3,4-dichlorobenzyl ether (21) and the isomeric 2,4-dichlorobenzyl ether (22) in the 2,6-dihydroxyphenyl ketone series were both only moderately potent inhibitors of aniline hydroxylase activity. Against aminopyrine demethylase the 2,4-dichlorobenzyl ether (22) was markedly more active than its isomer (21), perhaps because in 22, the relatively negative 2-chloro group is able to interact with the positive end of the carbonyl dipole. In the 2,4-dihydroxyphenyl ketone series only the 4-hydroxy group, which is remote from the carbonyl group, was

Table I. Inhibition of Aniline Hydroxylase Activity and Aminopyrine Demethylase Activity by Derivatives of 2,6-Dihydroxyphenyl Alkyl Ketones[a]



No.	R^1	R^2	R^3	R^4	R^5	R^6	$I_{50} \times 10^5$ M[b] AH	APDM
1	CH_3	H	H	H	H	H	39	32
2	H	OH	H	H	H	H	20	65
3	CH_3	OH	H	H	H	H	21	14
4	C_2H_5	OH	H	H	H	H	15	15
5	n-C_3H_7	OH	H	H	H	H	21	5
6	n-C_4H_9	OH	H	H	H	H	63	10
7	C_6H_5	OH	H	H	H	H	>107[c]	21
8	CH_3	OH	CH_3	H	H	H	11	17
9	CH_3	OH	C_2H_5	H	H	H	14	18
10	CH_3	OH	n-C_3H_7	H	H	H	18	19
11	CH_3	OH	n-C_4H_9	H	H	H	47	12
12	CH_3	OH	C_2H_5	H	CH_3CO	H	66	16
13	CH_3	OH	C_2H_5	H	C_2H_5	H	43	17
14	CH_3	OH	Br	H	Br	H	>53[c]	8
15	CH_3	OH	H	H	H	CH_3	20	9
16	CH_3	OH	H	H	H	C_2H_5	12	14
17	CH_3	OH	H	H	H	n-C_3H_7	14	15
18	CH_3	OH	H	H	H	n-C_4H_9	37	5
19	CH_3	OH	H	H	H	$CH_2=CHCH_2$	11	7
20	CH_3	OH	H	H	H	$C_6H_5CH_2$	19	8
21	CH_3	OH	H	H	H	3,4-$Cl_2C_6H_3CH_2$	70	31
22	CH_3	OH	H	H	H	2,4-$Cl_2C_6H_3CH_2$	45	6
23	CH_3	OH	H	CH_3	H	H	16	14
24	CH_3	OCH_3	H	H	H	CH_3	400	20
25	CH_3	OH	CH_3	H	H	CH_3	16	22
SKF 525-A							29	0.6
Piperonyl butoxide							42	42

[a] All data represent means of duplicate determinations which agreed within 15% of each other. [b] The control value of aniline hydroxylase (AH) activity was 2.22 ± 0.25 nmol of *p*-aminophenol formed/mg of protein/min and that for aminopyrine demethylase (APDM) activity was 1.51 ± 0.16 nmol of 4-aminoantipyrine formed/mg of protein/min. [c] Inhibition was less than 50% at this concentration, which was the approximate maximum solubility attainable.

etherified. In this series no difference between the potency of the two isomeric dichlorobenzyl ethers **43** and **44** as aminopyrine demethylase inhibitors was observed. Against aniline hydroxylase, with the exception of **41**, no inhibition was observed for any of these 4-alkoxy compounds (**37–44**). The replacement of the ether function by the ester group in 8-acetyl-7-hydroxy-4-methylcoumarin did not result in a startling loss of activity, I_{50}'s being 38×10^{-5} and 20×10^{-5} M toward aniline hydroxylase and aminopyrine demethylase, respectively.

Etherification of both hydroxyl groups was only carried out in one case. 2,6-Dimethoxyacetophenone (**24**) was essentially not an inhibitor of aniline hydroxylase. However, it gave reasonable inhibition of aminopyrine demethylase.

Interestingly, the 4-alkoxy-2-hydroxypropiophenones **37–44** contrast in their behavior to the 2,6-dioxygenated acetophenone monoethers **15-22**. The differences manifested can scarcely be accounted for by the extra carbon in the acyl group. Compound **37–44** are, with the exception of **41**, not inhibitors of aniline hydroxylase but possess similar inhibitory activity toward aminopyrine demethylase as compounds **15-22** and as the parent compound **28**.

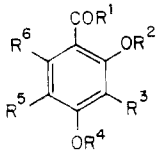
Ring Substituents. Introduction of alkyl groups into the 3 position of 2,6-dihydroxyacetophenone gave results somewhat similar to those found for the ethers insofar as inhibitory activity was greatest with a small substituent. Compounds **8–10** (Table I) were approximately as effective inhibitors of both oxidations as **3** (no substituent), but compound **11**, where the substituent is *n*-butyl, was relatively inactive against aniline hydroxylase activity but an effective aminopyrine demethylase inhibitor. 3,5-Diethyldihydroxyacetophenone (**13**) was also a poor inhibitor of aniline hydroxylase but effective against aminopyrine demethylase. In the 2,6-dihydroxyphenyl ketone series the 4-substituted derivatives were difficult to prepare. The only compounds readily accessible, 2,6-dihydroxy-4-methylacetophenone (**23**) and its methyl ether **25**, showed potencies similar to those of **3** and **15**, although **25** was distinctly less effective than **15** against aminopyrine demethylase.

The 5-alkyl-2,4-dihydroxyacetophenones **31–33** are analogues of **8–11**. Compound **31** is a good inhibitor of both enzyme systems, but increasing the length of the 5-alkyl group decreases activity analogously to changes in activity of **8–11**.

The only further compounds worth noting are the dibrominated ketones **14** and **36**. Both are poor inhibitors of aniline hydroxylase but were among the most potent inhibitors of aminopyrine demethylase prepared.

The effectiveness of some of these compounds as inhibitors of the MFO system in vivo was determined by measuring the prolongation of hexobarbital sleeping times and zoxazolamine paralysis times in mice (Table III). There was no apparent correlation between in vitro and in vivo data. Provided that a compound was effective in

Table II. Inhibition of Aniline Hydroxylase Activity and Aminopyrine Demethylase Activity by Derivatives of 2,4-Dihydroxyphenyl Alkyl Ketones[a]



No.	R1	R2	R3	R4	R5	R6	$I_{50} \times 10^5$ M[b] AH	APDM
26	H	H	H	H	H	H	140	110
27	CH_3	H	H	H	H	H	70	52
28	C_2H_5	H	H	H	H	H	75	16
29	n-C_3H_7	H	H	H	H	H	68	14
30	i-C_3H_7	H	H	H	H	H	60	14
31	CH_3	H	H	H	C_2H_5	H	6	7
32	CH_3	H	H	H	n-C_3H_7	H	17	8
33	CH_3	H	H	H	n-C_6H_{13}	H	22	40
34	C_2H_5	H	H	H	NO_2	H	>117[c]	100
35	n-C_3H_7	H	H	H	n-C_3H_7CO	H	ni[d]	17
36	C_2H_5	H	Br	H	Br	H	ni	6
37	C_2H_5	H	H	CH_3	H	H	ni	17
38	C_2H_5	H	H	C_2H_5	H	H	ni	18
39	C_2H_5	H	H	n-C_5H_{11}	H	H	ni	45
40	C_2H_5	H	H	n-C_8H_{17}	H	H	ni	>100[c]
41	C_2H_5	H	H	$C_2H_5OCH_2CH_2$	H	H	125	25
42	C_2H_5	H	H	$C_6H_5CH_2$	H	H	ni	75
43	C_2H_5	H	H	3,4-$Cl_2C_6H_3CH_2$	H	H	ni	29
44	C_2H_5	H	H	2,4-$Cl_2C_6H_3CH_2$	H	H	ni	31

[a] All data represent means of duplicate determinations which agreed within 15% of each other. [b] The control value of aniline hydroxylase (AH) activity was 2.22 ± 0.25 nmol of p-aminophenol formed/mg of protein/min and that for aminopyrine demethylase (APDM) activity was 1.51 ± 0.16 nmol of 4-aminoantipyrine formed/mg of protein/min. [c] Inhibition was less than 50% at this concentration, which was the approximate maximum solubility attainable. [d] No inhibition at concentrations of 10^{-3} M.

Table III. In Vivo Effects of Hydroxyphenyl Alkyl Ketones on Hexobarbital Sleeping Times and Zoxazolamine Paralysis Times in Mice

No.	% control, hexobarbital sleeping time[a,b]	% control, zoxazolamine paralysis time[a,b]
3	190	164
5	218	141
7	183	140
8	198	117[c]
10	164	94[c]
15	233	
19	162	191
20	170	156
24	128[c]	169
27	127[c]	77[c]
31		143

[a] Control values ± standard error (number of observations) were 55.2 ± 10.0 min (19) for hexobarbital sleeping time and 39.2 ± 5.8 min (16) for zoxazolamine paralysis time. [b] Unless otherwise noted all results were significantly different from control animals at $p = 0.01$. [c] Not significantly different from control animals at $p = 0.05$.

vitro, it also prolonged hexobarbital sleeping times. The maximum prolongation of sleeping times observed was about twofold while zoxazolamine paralysis times generally showed smaller increases over controls. Interestingly, compounds **8** and **10** prolonged hexobarbital activity but did not significantly affect zoxazolamine paralysis times. Compound **24** enhanced zoxazolamine activity but was not significantly effective in increasing hexobarbital sleeping times. Compound **27**, in agreement with its poor in vitro activity (Table II), was inactive in vivo.

Overall the data presented here do not allow any definitive conclusions to be drawn concerning structure–activity relationships among these inhibitors. Though this work appears to demonstrate an aniline hydroxylase inhibitory requirement of an o-hydroxyphenyl ketone, preliminary tests with 2,6-dihydroxyethylbenzene ($I_{50} = 45 \times 10^{-5}$ M) and the phenylhydrazone of **15** ($I_{50} = 10 \times 10^{-5}$ M) show that this need not be the case. It is notable that although the activity of the parent ketone **3** appears to be not readily improvable by molecular modification, activities found were comparable to those displayed by piperonyl butoxide and SKF 525-A (aniline hydroxylase only) (see Table I). Structural changes around the o-hydroxyaryl ketone system (Chart I) might be expected to lead to a loss of inhibitory potency since this portion of the molecule is presumably involved in binding the inhibitor to the MFO active site(s). The failure of substituents at positions 3, 4, and 5 of the phenyl ring to bring about any substantial improvement in inhibitory potency was disappointing. The MFO system is part of a lipoprotein complex[1] which would be expected to possess hydrophobic areas around the active site. If these were present then the introduction of suitable hydrophobic substituents into candidate inhibitors should lead to more potent compounds provided that the substituent is able to bind to such hydrophobic areas on the enzyme surface. In this series of ketones our attempts to prepare such compounds have led to loss of activity rather than any gain. That such hydrophobic areas do exist for aminopyrine demethylase and aniline hydroxylase has been shown with a series of alkyl-substituted benzimidazoles[9] where inhibition markedly increased with chain length of the alkyl group.

One interesting finding is the specificity of some of the candidate inhibitors for aminopyrine demethylase. This was shown by compounds **14**, **24**, and especially **35–39** and **42–44**. The reasons for this specificity are not yet clear. The MFO system is a mixture of oxidative enzymes, though the extent of multiplicity has not yet been resolved.[14,15] The results presented here clearly suggest that

Table IV. 6-Alkyl-7-hydroxy-4-methylcoumarins

No.	Substituent	Method[a]	Yield, %[b]	Mp, °C	Recrystn solvent	Formula	Analyses
45	C_2H_5	A	72	215-216	EtOH	$C_{12}H_{12}O_3$	C, H
46	n-C_3H_7	A	64	178-179	Aq EtOH	$C_{13}H_{14}O_3$	C, H
47	n-C_4H_9	A	93	161-162	Aq EtOH	$C_{14}H_{16}O_3$	C, H

[a] Letters relate to the general procedures given in the Experimental Section. [b] Final step yields of analytically pure compounds are given; no attempts were made to optimize the yields.

Table V. Preparation and Properties of Hydroxyphenyl Alkyl Ketones and Derivatives

No.	Method[a]	Yield, %[b]	Mp, °C	Recrystn solvent	Formula	Analyses	Ref (mp, °C)
2		50	154-155	H_2O	$C_7H_6O_3$		23 (155-156)
3	A	60	154-155	Aq EtOH	$C_8H_8O_3$		18 (154-155)
4	A	65	137-138	Acetone-hexane	$C_9H_{10}O_3$		18 (133-135)
5	A	59	116-117	Acetone-hexane	$C_{10}H_{12}O_3$		24 (116-118)
6	A	54	78-79	Acetone-hexane	$C_{11}H_{14}O_3$		25 (85-86)
7	A	50	134-136	H_2O	$C_{13}H_{10}O_3$		26 (135)
8	A	55	140-141	Acetone-hexane	$C_9H_{10}O_3$		27 (141)
9	A	60	130-131.5	Acetone-hexane	$C_{10}H_{12}O_3$	C, H	
10	A	64	84-85	Acetone-hexane	$C_{11}H_{14}O_3$	C, H	
11	A	70	72-73	Acetone-hexane	$C_{12}H_{16}O_3$	C, H	
15	B	73	60	Aq EtOH	$C_9H_{10}O_3$		28 (60)
16	B	65	85	Aq EtOH	$C_{10}H_{12}O_3$		22 (83-84)
17	B	80	72-73	Aq EtOH	$C_{11}H_{14}O_3$	C, H	
18	B	40	59-60	Aq EtOH	$C_{12}H_{16}O_3$	C, H	
19	B	82	54-55	EtOH	$C_{11}H_{12}O_3$	C, H	
20	B	57	110-111	EtOH	$C_{15}H_{14}O_3$		29 (109-110)
21	B	52	175-176	EtOH	$C_{15}H_{12}Cl_2O_3$	C, H, Cl	
22	B	62	126-127	EtOH	$C_{15}H_{12}Cl_2O_3$	C, H, Cl	
23		12	145-146	Acetone-hexane	$C_9H_{10}O_3$		30 (147)
25	B	80	57-58	Aq MeOH	$C_{10}H_{12}O_3$	C, H	
26		85	135	H_2O	$C_7H_6O_3$		31 (135-136)
27	C	54	149-150	Acetone-hexane	$C_8H_8O_3$		19 (142-144)
28	C	62	97-98	Acetone-hexane	$C_9H_{10}O_3$		21 (98-99)
29	C	57	68	Acetone-hexane	$C_{10}H_{12}O_3$		21 (68-70)
30	C	43	66	Acetone-hexane	$C_{10}H_{12}O_3$		27 (67-69)
31	C	82	117	Acetone-hexane	$C_{10}H_{12}O_3$		32 (118)
32	C	70	127	Acetone-hexane	$C_{11}H_{14}O_3$		32 (127-128)
33	C	68	89-90	Aq EtOH	$C_{14}H_{20}O_3$	C, H	
34		43	137-138	MeOH	$C_9H_9NO_5$		33 (131)
35	C[c]	5	64	Acetone-hexane	$C_{14}H_{18}O_4$		32 (64-65)
36	*d*	40	151-152	EtOH	$C_9H_{10}Br_2O_3$		21 (151-152)
37	B	40	55-57	Acetone-hexane	$C_{10}H_{12}O_3$		34 (55-56)
38	B	51	52-53	Acetone-hexane	$C_{11}H_{14}O_3$		35 (54)
39	B	64	*e*	*e*	$C_{14}H_{20}O_3$	C, H	
40	B	60	*e*	*e*	$C_{17}H_{26}O_3$	C, H	
41	B	74	45-46	*f*	$C_{13}H_{18}O_4$	C, H	
42	B	45	113-114	EtOH	$C_{16}H_{16}O_2$	C, H	
43	B	55	126-127	EtOH	$C_{16}H_{14}Cl_2O_3$	C, H, Cl	
44	B	43	109-110	EtOH	$C_{16}H_{14}Cl_2O_3$	C, H, Cl	

[a] Letters relate to the general procedures given in the Experimental Section. Absence of a letter indicates that the compound was prepared by the method used in the cited reference. [b] Final step yields of analytically pure compounds are given; no attempts were made to optimize the yields. [c] Isolated as the minor product from column chromatography on silica gel of crude 2,4-dihydroxybutyrophenone prepared by method C. [d] Described in the Experimental Section. [e] Viscous oil, purified by preparative TLC. [f] Purified by vacuum sublimation.

aniline hydroxylase and aminopyrine demethylase activities are mediated by different enzyme systems. It is probable that no single mechanism will account for the mode of action of all the ketones on the two enzymic activities measured. In vivo metabolic studies have shown that **24** undergoes O-demethylation to **15** as the principal metabolite and that substantial amounts of 2,6-dimethoxy-3-hydroxyacetophenone are also formed.[37] The latter arises from aromatic hydroxylation. It is probable that **24** is an alternative substrate for aminopyrine demethylase and indeed it appears to act as a competitive inhibitor.[12] The fact that **24** is a poor inhibitor for aniline hydroxylase in vitro while it is hydroxylated in vivo indicates that aniline and **24** are substrates for different enzymes. In vivo **2** does not undergo oxidative metabolism and **15** is oxidized only slightly.[37]

Experimental Section

Preparation of Microsomal Fraction. White, male Wistar derived rats (100–150 g) were given phenobarbitone (60 mg/kg) by ip injection daily for 3 days. They were killed on the fourth day; their livers were removed, rinsed with potassium phosphate buffer (0.1 M, pH 7.4), weighed, and homogenized (Waring blender) in 7 vol of buffer. The homogenate was centrifuged at 1000g for 10 min and then at 9000 g for 10 min. The resulting supernatant fraction was then centrifuged at 105000 g for 60 min. The microsomal pellet was resuspended in sufficient 0.1 M phosphate buffer, pH 7.4, so that 1 mL contained microsomal protein derived from 1 g of liver. Protein was determined by the method of Robinson and Hodgson.[16]

Enzyme Assays. For aniline hydroxylase the final solution (3.5 mL) contained aniline (15 μmol), $MgCl_2$ (7.5 μmol), NADP (2.5 μmol), glucose 6-phosphate (10 μmol), glucose-6-phosphate dehydrogenase (2 units), microsomal protein (8.6 mg), and

phosphate buffer, pH 7.4 (0.35 mmol). Incubation was carried out at 37 °C for 10 min and *p*-aminophenol was assayed according to Gilbert and Goldberg.[17] In some cases the inhibitor interfered with the assay. Where this occurred the supernatant from the TCA precipitate was extracted with ether, the ethereal layer discarded, and an aliquot of the aqueous layer assayed.

Aminopyrine demethylase incubations were carried out with cofactors as for aniline hydroxylase. The solution (3.0 mL) contained aminopyrine (1.5 μmol), microsomal protein (5.6 mg), and phosphate buffer, pH 7.4 (0.3 mmol). Incubation time was 20 min and the extent of reaction was determined by assaying for 4-aminoantipyrine.[17] This was chosen rather than the formaldehyde assay because of inhibitor interference in the Nash reaction.

Inhibitors were usually added in aqueous solution. The benzyl ethers **20–22** and **42–44** were added in ethanol (100 μL) for aniline hydroxylase or dimethyl sulfoxide (100 μL) for aminopyrine demethylase. In each case the addition of solvent alone resulted in a 30% inhibition of enzyme activity. I_{50} values were determined using a range of five to seven inhibitor concentrations and compared with those obtained for SKF 525-A and piperonyl butoxide.

In Vivo Inhibition of Hexobarbitone and Zoxazolamine Metabolism. Male white mice (25–30 g) were given inhibitors (100 mg/kg) in 25–50% aqueous macrogol 300 by ip injection. After 10 min hexobarbital or zoxazolamine (100 mg/kg) was injected. Sleeping or paralysis time was taken from the loss of righting reflex until it was completely regained. Mice were kept at 35–37 °C during the experiments. The macrogol solutions used as vehicle did not significantly alter sleeping or paralysis times from those of untreated control animals.

Synthesis. All compounds prepared in this work gave IR and NMR spectra in agreement with their assigned structures and were homogeneous by TLC. New compounds gave elemental analyses within ±0.4% of the calculated values.

2,6-Dihydroxyphenyl Alkyl Ketones (Method A). These were prepared by the method of Russel and Frye[18] for 2,6-dihydroxyacetophenone. Intermediate 7-hydroxy-4-methylcoumarins were purified and had physical properties in agreement with literature values. New coumarins are listed in Table IV. Esterified coumarins were characterized by NMR and IR spectra but not by elemental analysis. These were used for the Fries rearrangement and the crude product from this immediately hydrolyzed with alkali to the dihydroxyphenyl ketone. Where necessary these were purified by chromatography on silica gel (100–200 mesh). The properties of compounds **2–10** prepared by this method are summarized in Table V.

2-Hydroxy-6-methoxy-3-methylacetophenone (25) (Method B). 2,6-Dihydroxy-3-methylacetophenone (**8**) (0.66 mmol) and CH_3I (1.2 mmol) in acetone (2 mL) were refluxed in the presence of anhydrous K_2CO_3 (0.72 mmol) for 5 h, diluted with water, and extracted with ether. The ethereal solution was washed with 20% NaOH solution to isolate the phenolic fraction which was sublimed in vacuo. The sublimate was crystallized from aqueous CH_3OH affording an 80% yield: mp 57–58 °C. Anal. ($C_{10}H_{12}O_3$) C, H. The structure was assigned by NMR decoupling studies. Absorptions for **25** were found at δ 2.19 (s, 3, 3-CH_3), 2.68 (s, 3, CH_3CO), 3.81 (s, 3, 6-OCH_3), 6.27 (d, J = 8 H_z, 1, 5-H), 7.18 (d, J = 8 Hz, 1, 4-H), and 13.65 (s, 1, 2-OH) at 60 mHz. At 100-mHz the absorption at δ 2.19 (3-CH_3) split into a doublet (J = 0.9 Hz). Irradiation at δ 7.8 (4-H) gave a singlet at δ 2.19 but did not affect the methoxyl resonance at δ 3.81. Irradiation at δ 6.27 (5-H) considerably sharpened the methoxyl proton signal but did not affect that of the *C*-methyl group, confirming the assigned structure.

The properties of other compounds (**15–22, 37–44**) prepared by this method are summarized in Table V.

2,4-Dihydroxyphenyl Alkyl Ketones (Method C). These were prepared by the method of Cooper[19] for 2,4-dihydroxyacetophenone (**27**). The crude products were purified by chromatography on silica gel using 8% acetone in hexane as eluent. The properties of compounds **27–33** prepared in this way are summarized in Table V.

4-Alkylresorcinols required for the synthesis of compounds **8–11** were prepared by Clemmenson reduction of the appropriate carbonyl compound. Their properties agreed with those previously reported in the literature.

3,5-Diethyl-2,6-dihydroxyacetophenone (13) and 1,3-Diacetyl-5-ethyl-2,6-dihydroxybenzene (12). 4,6-Diethylresorcinol[20] (5 mmol) was refluxed with acetic anhydride (20 mmol) in pyridine (5 mL) for 2 h. After dilution with water the oily product was extracted into ether, washed with dilute HCl, water, and 5% $NaHCO_3$. Removal of the ether gave an oil (95% yield) which was homogenous by TLC. IR and NMR spectra confirmed its structure as 4,6-diethylresorcinol diacetate. The diacetate (2.7 mmol) and anhydrous $AlCl_3$ (6 mmol) were heated at 155 °C for 1 h, cooled, and then warmed with dilute HCl. The residual solid was dissolved in 5% aqueous NaOH, filtered, and reprecipated. Separation by preparative TLC (acetone–hexane, 1:4 v/v) afforded **12** [yield 57%; mp 71–73 °C from acetone–petroleum ether (lit.[20] mp 72 °C)] and **13** (yield 15%; mp 68–70 °C from acetone–petroleum ether). Anal. ($C_{12}H_{16}O_3$) C, H.

2,6-Dihydroxy-3,5-dibromoacetophenone (14). A solution of **2** (13 mmol) in acetic acid (20 mL) was treated with Br_2 (26 mmol) in acetic acid (4 mL). After standing for 2 h dilution with water gave a solid: mp 171–172 °C from ethanol; yield 60%. Anal. ($C_8H_6Br_2O_3$) C, H, Br. 3,5-Dibromo-2,4-dihydroxypropiophenone (**36**) was prepared similarly.

2,6-Dimethoxyacetophenone (24). To a refluxing solution of 2,6-dihydroxyacetophenone (7.9 mmol) in ethanol (5 mL), $(CH_3)_2SO_4$ (25 mmol) in 10 M NaOH (2 mL) was added dropwise. The solution was made alkaline with 10 M NaOH and refluxed for 5 h, diluted with water, and extracted with ether. The ethereal solution was washed with dilute NaOH and water and dried. Evaporation of the ether gave a solid: mp 72–73 °C from water; yield 71% (lit.[22] mp 72–73 °C).

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Oxime Ether Derivatives, a New Class of Nonsteroidal Antiinflammatory Compounds

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A series of new 2-hydroxyethyl and carboxyalkyl ethers of aromatic oximes was found to possess pronounced antiinflammatory activity in the carrageenan-induced edema test in the rat. The activity was limited mainly to derivatives of *p*-haloacetophenone oxime and of *p*-halobenzaldehyde oxime. Nevertheless, the hydroxyethyl and carboxyalkyl groups may be converted into many derivatives with maintenance of activity. Some structure–activity relationships are in contrast to those of the well-known antiinflammatory arylacetic acids. The activity is limited to the *E* stereoisomers. The hydrochloride of 2-(dimethylamino)ethyl (*E*)-[[(*p*-chloro-α-methylbenzylidene)-amino]oxy]acetate (**36**, INN name Cloximate) was chosen for clinical evaluation. The first results agree with the pharmacological prospects.

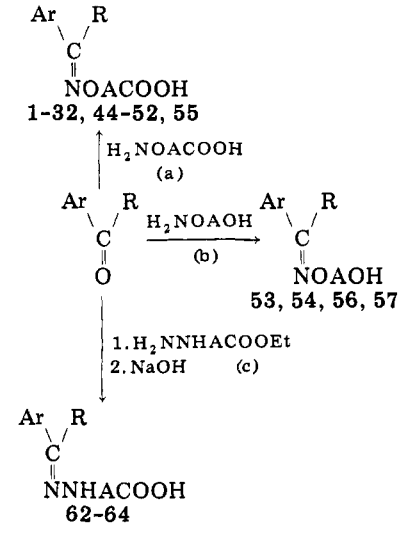
In the course of pharmacological investigations of different types of oxime ethers,[1] we found *p*-chloroacetophenone oxime ether of acetic acid (**7**) to possess a pronounced antiinflammatory activity (AIA) and favorable toxicity. A great number of analogues were synthesized and investigated with respect to this activity. This paper describes the syntheses of the compounds and structure–activity relations (SAR) are discussed.

Chemistry. As early as 1896 the first oxime acetic acid and its ethyl ester were synthesized from benzaldehyde oxime and chloroacetic acid.[2] At the same time a synthesis of aminooxyacetic acid was described,[3] which compound was used some 40 years later for isolation procedures, etc., of ketones.[4,5] Limited groups of oxime ethers of alkanoic acids have since then been prepared by Richardson[6] and recently by Buzas et al.[7]

As indicated in Schemes I and II we used ketones (and aldehydes) as well as the oximes for the preparation of our oxime ethers. The condensation of a ketone with a hydroxylamino ether (Scheme I) was the most convenient way of making oxime ethers of a large series of variably substituted aromatic ketones and aldehydes. For the preparation of oxime ethers composed of variable acids linked with a few preferable oximes, the methods of Scheme II were chosen, which were variations of known conversions.

An interesting phenomenon was the fact that with phenyl ketones always the *E* oximes or *E* oxime ethers were obtained in large excess, whereas analogous thienyl derivatives gave both stereoisomers, *E* and *Z*, in considerable amounts. The reason might be sought in steric effects, i.e., the influence of the absence of one "ortho" substituent in the α-thienyl derivatives. The amount of *Z* isomer found in **2** and **48** (respectively 9 and 6% of the theoretical yield), which seems in contradiction with this explanation, might be a coincidence (N.B. **23** contained no *Z* isomer). The amount formed seems rather small and cannot be conclusive.

Scheme I. Conversions of Ketones and Aldehydes[a]



[a] A = alkylene; Ar = (substituted) aryl or heteroaryl; R = mainly H or Me; numbers, see Table I; letters in parentheses, see Experimental Section under methods.

Different types of the oxime ethers were easily obtainable from others, without isomerization or instability of the oxime ether function being observed (Scheme III). For instance, anhydrous (alcoholic) acid leads from acids to esters quantitatively (Scheme III, method j). Acid chlorides are formed with $SOCl_2$ (k), and these are converted into esters with additional functional groups, e.g., into basic esters (m). Only with anhydrous HCl in ether an *E* isomer is isomerized to the *Z* isomer (n). In an aqueous acidic medium the oxime ether is slowly hydrolyzed to the hydroxylamino ether and the ketone (p). In alkaline conditions the oxime ether is stable as is shown by the formation of the oxime ethers from the oximate ion (Scheme II) and in the formation of oxime ether amides