were used to determine the mean lethal concentration (CE_{37}), defined as the concentration required to reduce cloning efficiency to a factor of 0.37.

Acknowledgment. We are very grateful to J. Couprie, S. Guérineau, and D. Murphy for their technical assistance and to Dr. J. Feunteun who generously supplied the *E. coli* bacterial strain containing the pUC13 plasmid. This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut de la Santé et de la Recherche Médicale, the Université Pierre et Marie Curie (Paris VI), the Université René Descartes (Paris V), and the Association pour le Développement de la Recherche sur le Cancer (ARC, Villejuif).

Registry No. 1, 102852-77-7; 2, 102852-78-8; 3, 102852-79-9; 4, 239-13-4; 5, 102852-59-5; 6, 102852-60-8; 7, 239-03-2; 8, 102852-61-9; 9, 239-06-5; 10, 102852-62-0; 11, 102852-63-1; 12, 53645-54-8; 13, 102852-64-2; 14, 102852-65-3; 15a, 102852-80-2; 15b, 102852-81-3; 16a, 76402-00-1; 16b, 102852-82-4; 17, 102852-66-4; 18, 102852-67-5; 19, 102852-68-6; 20, 102852-69-7; 21, 102852-70-0; 22, 102852-71-1; 23, 102852-72-2; 24, 102852-73-3; 25, 102852-74-4; 26, 102852-75-5; 27, 102852-76-6; 8-quinolylhydrazine dihydrochloride, 91004-61-4; 4-methoxycyclohexanone, 13482-23-0; 4-methoxycyclohexanone 8-quinolylhydrazone, 102852-55-1; 5-isoquinolylhydrazine dihydrochloride, 102852-56-2; 4-methoxycyclohexanone 5-isoquinolylhydrazone, 102852-57-3; 5-quinolylhydrazine dihydrochloride, 91004-60-3; 4-methoxycyclohexanone 5-quinolylhydrazone, 102852-58-4; 3-formylindole, 487-89-8; 4-ethylpyridine, 536-75-4; 5-methoxy-3-formylindole, 10601-19-1; 2-methylellipticinium acetate, 69492-82-6; 2-methyl-9-hydroxyellepticinium acetate, 58337-35-2; 2-methyl-10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium iodide, 62099-82-5; 2-methyl-10-hydroxy-7*H*-pyrido[4,3-*c*]carabazolium iodide, 62099-85-8.

Comparative Toxicities and Analgesic Activities of Three Monomethylated Analogues of Acetaminophen

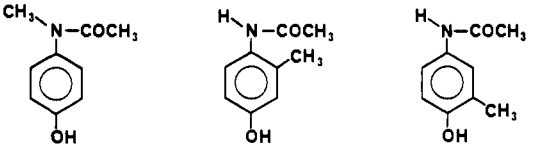
Peter J. Harvison, Anthony J. Forte, and Sidney D. Nelson*

Department of Medicinal Chemistry, School of Pharmacy, University of Washington, BG-20, Seattle, Washington 98195.
Received August 26, 1985

Three monomethylated derivatives of 4'-hydroxyacetanilide (acetaminophen) were prepared in order to compare their cytotoxic potential and analgesic activity with that of acetaminophen. Only 4'-hydroxy-*N*-methylacetanilide (*N*-methylacetaminophen) was devoid of cytotoxic effects to hepatic tissue of mice. Results of comparative tissue distribution studies and metabolism studies both in vivo and in vitro in mice indicate that the disposition of *N*-methylacetaminophen is similar to that of acetaminophen except that it is not oxidized to a toxic metabolite. In contrast, 3'-methyl-4'-hydroxyacetanilide (3-methylacetaminophen) is as hepatotoxic as acetaminophen in mice while 2'-methyl-4'-hydroxyacetanilide (2-methylacetaminophen) is less hepatotoxic. The analgesic potency of the analogues seems to parallel their hepatotoxic potential, and both activities parallel the oxidation potentials in this series of compounds.

The widely used analgesic drug, acetaminophen (**1**), is known to cause serious liver necrosis at high doses in man and experimental animals.[1] An electrophilic product of cytochrome P-450 oxidation that depletes cellular glutathione (GSH) and covalently binds to tissue macromolecules has been implicated in this toxic reaction.[2] Initially, the formation of the arylating metabolite was believed to involve N-oxidation of acetaminophen to *N*-hydroxyacetaminophen (**3**) followed by dehydration to *N*-acetyl-*p*-benzoquinone imine (NAPQI, **2**, Figure 1).[3] *N*-Hydroxyacetaminophen was synthesized in crystalline form and found to possess both chemical and toxicological characteristics that were consistent with its postulated role as an intermediate in the metabolism of acetaminophen to an arylating metabolite.[4] However, subsequent studies have shown that if *N*-hydroxyacetaminophen is an intermediate in microsomal oxygenase-mediated covalent binding of acetaminophen to tissue protein, it must decompose at the enzymatic site of hydroxylation.[5] NAPQI, on the other hand, has been recently detected as a cytochrome P-450 catalyzed oxidation product of acetaminophen in reactions that utilize cumene hydroperoxide, and NAPQI has been found to be highly cytotoxic.[6]

Inasmuch as N-oxidation and attack by tissue nucleophiles on the oxidized aromatic ring of acetaminophen are features that correlate with hepatotoxicity, we prepared the three monomethylated analogues (**4**–**6**) with the expectation that N-oxidation and/or reaction with tissue nucleohpiles would be hindered. We have previously documented that the *N*-methyl analogue of acetaminophen was not hepatotoxic or nephrotoxic in animal models for these toxic reactions caused by acetaminophen.[7] We now



4 **5** **6**

(1) (a) Prescott, L. F. *Drugs* **1983**, *25*, 290. (b) Hinson, J. A. In *Reviews in Biochemical Toxicology*; Hodgson, F., Bend, J. R., Philpot, R. M., Eds.; Elsevier/North-Holland: New York, 1980; Vol. 2, p 103.
(2) Mitchell, J. R.; Jollow, D. J.; Potter, W. Z.; Gillette, J. R.; Brodie, B. B. *J. Pharmacol. Exp. Ther.* **1973**, *187*, 211.
(3) Jollow, D. J.; Thorgeirsson, S. S.; Potter, W. Z.; Hashimoto, M.; Mitchell, J. R. *Pharmacology* **1974**, *12*, 251.
(4) (a) Healey, K.; Calder, I. C.; Yong, A. C.; Crowe, C. A.; Funder, C. C.; Ham, K. N.; Tange, J. D. *Xenobiotica* **1978**, *8*, 403. (b) Gemborys, M. W.; Gribble, G. W.; Mudge, G. H. *J. Med. Chem.* **1978**, *21*, 649. (c) Calder, I. C.; Healey, K. *Aust. J. Chem.* **1979**, *32*, 1307. (d) Corcoran, G. B.; Mitchell, J. R.; Vaishnav, Y. N.; Horning, E. C. *Mol. Pharmacol.* **1980**, *18*, 536.
(5) Hinson, J. A.; Pohl, L. R.; Gillette, J. R. *Life Sci.* **1979**, *24*, 2133; Nelson, S. D.; Forte, A. J.; Dahlin, D. C. *Biochem. Pharmacol.* **1980**, *29*, 1617.
(6) (a) Dahlin, D. C.; Nelson, S. D. *J. Med. Chem.* **1982**, *25*, 885. (b) Dahlin, D. C.; Miwa, G. T.; Lu, A. Y. H.; Nelson, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1327. (c) Holme, J. A.; Dahlin, D. C.; Nelson, S. D.; Dybing, E. *Biochem. Pharmacol.* **1984**, *33*, 401.
(7) Nelson, S. D.; Forte, A. J.; McMurty, R. J. *Res. Commun. Chem. Pathol. Pharmacol.* **1978**, *22*, 61.

0022-2623/86/1829-1737$01.50/0

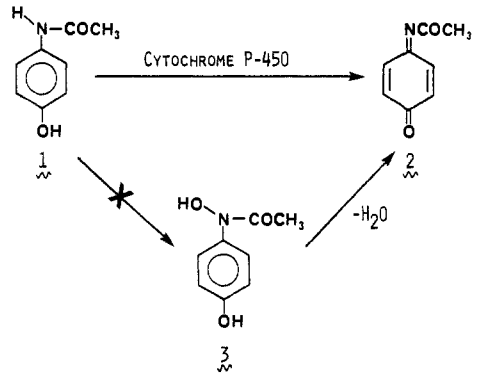


Figure 1. Possible pathways for the metabolism of acetaminophen (1) to *N*-acetyl-*p*-benzoquinone imine (2).

Table I. Redox Potentials of Acetaminophen and Its Monomethyl Analogues[a]

compd	redox potential, V, at given pH					
	0	3	6	7.4	9	12
acetaminophen	0.72	0.61	0.46	0.39	0.33	0.22
N-methylacetaminophen	0.84	0.78	0.74	0.68	0.63	0.53
2-methylacetaminophen	0.75	0.63	0.54	0.45	0.39	0.26
3-methylacetaminophen	0.67	0.53	0.43	0.36	0.28	0.10

[a] Half-wave potentials (V) were determined at a sweep rate of 150 mV/s from the first sweep of the oxidation wave of cyclic voltammograms. Solutions (0.2 M ionic strength) were 1 mM in acetaminophen or its analogues and were degassed with N_2 before running the scan. Scans were initiated at 0.0 V in the positive direction. Spectra were recorded with a glassy carbon electrode with a reference electrode, Ag/AgCl/3 M NaCl.

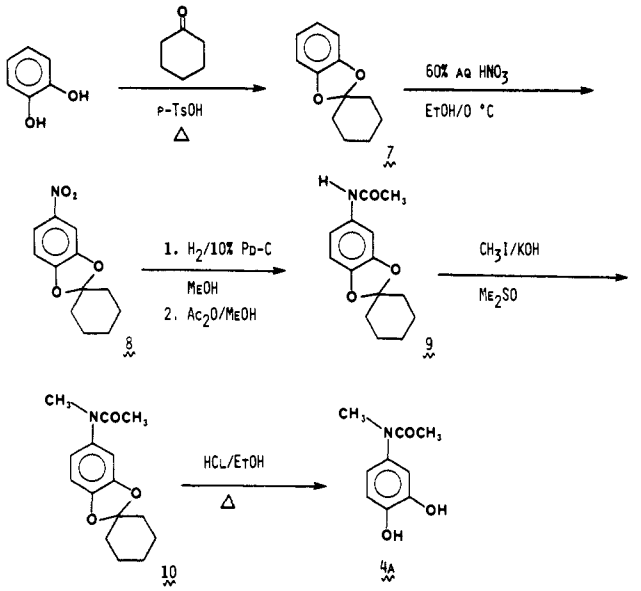
report results of metabolism studies in mice comparing the disposition of acetaminophen and *N*-methylacetaminophen. We also describe results of toxicity tests in mice with 2- and 3-methylacetaminophen and comparisons of the analgesic activity of acetaminophen and the monomethylated analogues.

Results

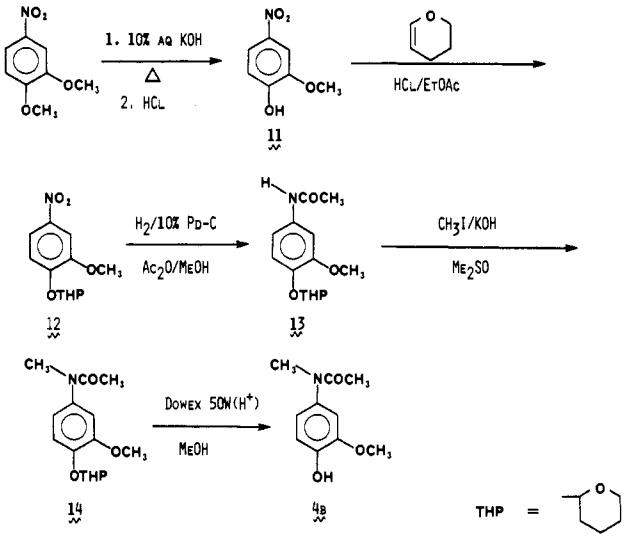
Chemistry. *N*-Methylacetaminophen and *N*-[acetyl-*1*-^{14}C]-4′-hydroxy-*N*-methylaniline ([^{14}C]-*N*-methylacetaminophen) were synthesized essentially by the method of Fernando et al.[8] by acylation of 4-(methylamino)phenol sulfate with either acetic anhydride or [1-^{14}C]acetic anhydride. Ring-methylated analogues of acetaminophen were prepared by modifying procedures described in the literature[9] as described in the Experimental Section. Two two metabolites, 3′,4′-dihydroxy-*N*-methylacetanilide (**4a**) and 3′-methoxy-4′-hydroxy-*N*-methylacetanilide (**4b**) were synthesized from protected catechol derivatives. Metabolite **4a** was prepared by N-methylation of the cyclohexanone ketal of 3′,4′-dihydroxyacetanilide (**9**), which was prepared as shown in Scheme I from the protected catechol (**7**). Metabolite **4b** was prepared in a similar manner (Scheme II) from the tetrahydropyranyl ether of 3-methoxy-4-hydroxynitrobenzene (**12**).

Oxidation potentials (Table I) were determined as half-wave potentials of the first oxidation wave recorded during cyclic voltammetric scanning. A more complete analysis of the cyclic voltammograms will appear in a future publication. At all pHs *N*-methylacetaminophen had the highest potential. At pHs near neutrality acetaminophen and 3-methylacetaminophen were oxidized at similar

Scheme I



Scheme II



potentials, whereas 2-methylacetaminophen was oxidized at higher potentials.

Hepatotoxicity. Because of an infection of the Swiss-Webster mouse colony that we had used for our previous studies,[7] we carried out additional studies to compare the hepatotoxic effects of acetaminophen and *N*-methylacetaminophen in Swiss-Webster mice from a new source. Although mice from the new source were significantly more sensitive to the hepatotoxic effects of acetaminophen, *N*-methylacetaminophen still did not cause hepatotoxicity in these mice as indicated by the lack of necrotic foci in the liver and lack of rise in plasma ALT concentrations (Table II). Deaths in the *N*-methylacetaminophen treated mice appeared to result primarily from the convulsive effects of the drug.

Both 2- and 3-methylacetaminophen were hepatotoxic to mice (Table II). Whereas 2-methylacetaminophen was significantly less hepatotoxic than acetaminophen ($p < 0.01$ at 750 mg/kg based on both mean necrosis scores and plasma ALT concentrations), 3-methylacetaminophen was not significantly less hepatotoxic. As with acetaminophen[10]

(8) Fernando, C. R.; Calder, J. C.; Ham, K. N. *J. Med. Chem.* **1980**, *23*, 1153.
(9) (a) Proskouiakoff, A.; Titherington, R. J. *J. Am. Chem. Soc.* **1930**, *52*, 3978. (b) Albert, H. E. *J. Am. Chem. Soc.* **1954**, *76*, 4985.
(10) Mitchell, J. R.; Jollow, D. J.; Potter, W. Z.; Davis, D. C.; Gillette, J. R.; Brodie, B. B. *J. Pharmacol. Exp. Ther.* **1973**, *187*, 185.

Table II. Comparison of Hepatoxicity in Swiss-Webster Mice Caused by Acetaminophen and Its Monomethyl Analogues

compd	dose, mg/kg, ip	survivors at 24 h	hepatic necrosis in survivors	plasma ALT at 24 h,[a] mU/mL
acetamino-phen	400	8/10	1.13 ± 0.35	1552 ± 501
	750	4/10	3.25 ± 0.25	4030 ± 829
N-methyl-acetamino-phen	500	9/10	0	27 ± 9
	1000	5/10	0	32 ± 8
2-methylacet-aminophen	750	10/10	0.80 ± 0.25	690 ± 189
	1000	8/10	1.75 ± 0.37	2113 ± 437
	1000[b]	5/10	3.40 ± 0.55	3920 ± 714
	1000[c]	9/10	0	40 ± 6
	1000[d]	10/10	0	75 ± 32
3-methylacet-aminophen	400	9/10	1.00 ± 0.33	1078 ± 350
	750	4/10	2.75 ± 0.25	3251 ± 905
	400[b]	4/10	3.50 ± 0.29	5127 ± 1310
	400[c]	8/10	0.56 ± 0.26	345 ± 209
	400[d]	9/10	0	83 ± 71

[a] Values are means ± SE. [b] Phenobarbital pretreated animals. [c] Cobaltous chloride pretreated animals. [d] Piperonyl butoxide pretreated animals.

phenobarbital pretreatment of mice increased the extent of centrilobular necrosis that was caused by both 2- and 3-methylacetaminophen, whereas pretreatment with either cobaltous chloride or piperonyl butoxide decreased the extent of liver necrosis that was caused by the two analogues. These results indicate that metabolism by hepatic microsomal oxygenases is required for manifestation of hepatotoxicity caused by 2- and 3-methylacetaminophen. It should be noted that deaths in mice treated with acetaminophen and its 2- and 3-methyl analogues were deaths that occurred between 10 and 24 h, and most likely are related to hepatotoxic effects of the drugs. No convulsive effects were observed.

Additional studies of GSH depletion further implicated reactive metabolite formation in the hepatotoxic reaction, since hepatic GSH was depleted more significantly by those analogues that caused the most extensive hepatic necrosis. Thus, the time course and extent of GSH depletion was similar for acetaminophen and 3-methylacetaminophen and slower and less extensive with the 2-methyl analogue (Figure 2). In contrast, *N*-methylacetaminophen only slightly affected hepatic GSH status.

Distribution and Metabolism of *N*-Methylacetaminophen. The lack of hepatotoxic effects of *N*-methylacetaminophen compared with acetaminophen could be a result of differences in absorption, distribution, and/or metabolism. At doses of 3 mmol/kg of [*acetyl*-^{14}C]acetaminophen and [*acetyl*-^{14}C]-*N*-methylacetaminophen the distribution of radioactivity over time was very

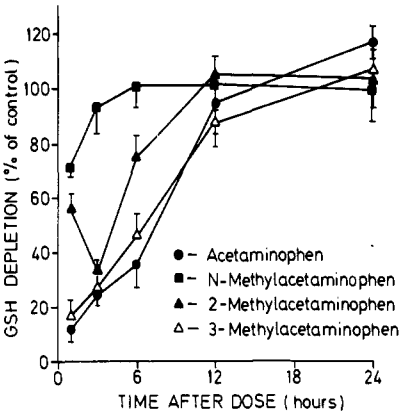


Figure 2. Time course of liver GSH depletion in male Swiss-Webster mice after the ip administration of 5 mmol/kg of acetaminophen and its monomethylated analogues.

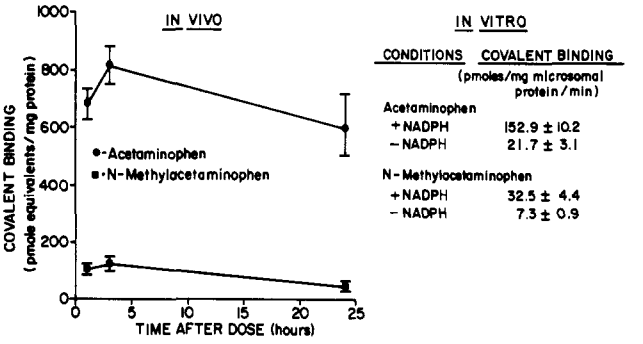


Figure 3. Time course of covalent binding of [*acetyl*-^{14}C]acetaminophen and [*acetyl*-^{14}C]-*N*-methylacetaminophen to mouse hepatic tissue. Each point is the mean of four determinations, and error bars indicate standard deviations.

similar to most mouse tissue (Table III). In particular, at early time points distribution of radiolabel to the liver was virtually equivalent. However, 24 h after the dose, residual radioactivity in the liver was greater in mice treated with radiolabeled acetaminophen compared to radiolabeled *N*-methylacetaminophen.

HPLC analysis of β-glucuronidase/sulfatase hydrolyzed samples of homogenized mouse liver showed that 91 ± 6% of the radioactivity that was present in the liver 1 h after a dose of acetaminophen was recovered as acetaminophen. Similarly, 87 ± 8% of the radioactivity in hydrolyzed liver samples of mice was recovered as *N*-methylacetaminophen 1 h after treatment with radiolabeled *N*-methylacetaminophen. On the other hand, the urinary spectrum of metabolites 24 h after doses of acetaminophen and *N*-methylacetaminophen in mice was quite different (Table

Table III. Time Course of Distribution of Radioactivity in Mice after 3 mmol/kg Doses of Radiolabeled Acetaminophen and *N*-Methylacetaminophen[a]

	time after dose, h					
	1		3		24	
tissue	acetaminophen	*N*-methylacetaminophen	acetaminophen	*N*-methylacetaminophen	acetaminophen	*N*-methylacetaminophen
brain	1.22 ± 0.10	1.03 ± 0.20	0.24 ± 0.06	0.05 ± 0.02	0.05 ± 0.01	0.01 ± 0.01
liver	2.89 ± 0.25	3.16 ± 0.78	0.73 ± 0.20	0.60 ± 0.05	0.58 ± 0.18	0.05 ± 0.02
kidney	2.45 ± 0.19	3.73 ± 0.78	1.19 ± 0.15	0.49 ± 0.09	0.21 ± 0.08	0.05 ± 0.01
stomach	1.13 ± 0.03	0.21 ± 0.61	0.71 ± 0.03	0.87 ± 0.19	0.15 ± 0.01	0.08 ± 0.04
intestines	2.19 ± 0.18	2.17 ± 0.21	1.08 ± 0.35	1.52 ± 0.39	0.92 ± 0.27	0.61 ± 0.36
spleen	1.55 ± 0.58	1.58 ± 0.54	0.29 ± 0.04	0.13 ± 0.11	0.04 ± 0.02	0.03 ± 0.02
lungs/heart	1.13 ± 0.20	1.78 ± 0.23	0.20 ± 0.07	0.13 ± 0.04	0.11 ± 0.02	0.03 ± 0.01
blood	1605 ± 88.0	1855 ± 116	544 ± 10.5	501 ± 22.4	19.2 ± 6.10	14.6 ± 3.95
urine	12.4 ± 2.60	7.67 ± 8.61	3880 ± 910	3200 ± 302	6005 ± 1490	6978 ± 2817
feces	0.09 ± 0.13	0.03 ± 0.04	1.52 ± 0.83	3.17 ± 3.10	15.3 ± 6.66	74.5 ± 20.8

[a] Values represent means ± SE (N = 4) and are expressed as nmol equiv/mg of tissue or nmol equiv/mL of blood or urine.

Table IV. Comparative Urinary Metabolic Profiles of Acetaminophen and N-Methylacetaminophen in the Mouse 24 h after Doses of 3 mmol/kg of Each Drug

compd	metabolite[a]	% admin dose[b]
acetaminophen	acetaminophen	68.8 ± 4.2
	3-hydroxyacetaminophen	0.4 ± 0.2
	3-methoxyacetaminophen	1.1 ± 0.3
	3-cysteinylacetaminophen	18.5 ± 1.4
	3-(methylthio)acetaminophen	2.3 ± 0.8
	acetaminophen-3-mercapturate	2.0 ± 0.4
N-methylacetaminophen	N-methylacetaminophen	78.9 ± 5.0
	acetaminophen	1.9 ± 0.5
	3-hydroxy-N-methylacetaminophen	0.6 ± 0.4
	3-methoxy-N-methylacetaminophen	13.7 ± 1.9

[a] Determined by HPLC as described in the Experimental Section after hydrolysis of urine samples with β-glucuronidase/sulfatase. [b] Results are reported as means ± SE (N = 4).

Table V. Analgesic Activity of Acetaminophen and Its Monomethylated Analogues as Determined in Mice Using the Bradykinin-Induced Writhing Test

compd	analgesic ED_{50},[a] mg/kg
acetaminophen	84.3 (27–108)
N-methylacetaminophen	>200
2-methylacetaminophen	177.1 (138–195)
3-methylacetaminophen	94.6 (77–116)

[a] Numbers in parentheses are 95% SE limits as obtained from Probit analysis.

IV). Although over 70% of the administered dose was recovered in the urine after 24 h as free drug plus glucuronide and sulfate conjugates in each case, the remainder of the acetaminophen dose was largely excreted as sulfur–ether conjugates, reflecting reactive metabolite formation. In contrast, the remainder of the dose of N-methylacetaminophen was largely excreted as catechol metabolites.

Covalent (irreversible) binding of radiolabel from [*acetyl*-^{14}C]acetaminophen and [*acetyl*-^{14}C]-N-methylacetaminophen to mouse liver protein in vivo and to microsomes prepared from mouse liver (Figure 2) further demonstrated that acetaminophen is metabolized extensively to reactive metabolites that bind to tissue protein, whereas N-methylacetaminophen is transformed much less extensively to metabolites that covalently bind to tissue macromolecules. Although some binding may simply reflect acetyl group transfer, it has been established for acetaminophen that approximately 80% of the binding to mouse liver protein involves the entire hydroxyacetanilide structure.[11]

Analgesic Activity of Acetaminophen and Its Monomethylated Analogues. Analgesic activities were compared (Table V) for acetaminophen and its monomethylated analogues in mice by determining ED_{50} values for the inhibition of bradykinin-induced writhing.[12] Acetaminophen and 3-methylacetaminophen were approximately equipotent, whereas 2-methylacetaminophen was significantly less potent. N-methylacetaminophen was inactive in this test.

Discussion

Hepatotoxicity after ingestion of large doses of acetaminophen is caused by a metabolic oxidation product of the drug that is most likely NAPQI, 2. The results reported in this paper support this conclusion inasmuch as N-methylation of acetaminophen completely blocks the hepatotoxic reaction, even though this modification yields an analogue that has similar dispositional characteristics to acetaminophen. Metabolism of N-methylacetaminophen in mice is also similar to that of acetaminophen except for the lack of detectable formation of thio–ether metabolites.

In contrast, two regioisomeric aryl methylated analogues of acetaminophen are hepatotoxic in mice. Interestingly, 3-methylacetaminophen is as hepatotoxic as acetaminophen, while 2-methylacetaminophen is significantly less hepatotoxic. These results parallel results of toxicity studies with dimethylated analogues of acetaminophen.[8] Methyl groups ortho to the phenolic site (3,5-dimethylacetaminophen) were found to have no effect on hepatotoxicity when compared to acetaminophen, while methyl groups ortho to the amide function (2,6-dimethylacetaminophen) were found to block hepatotoxicity. Effects of cytochrome P-450 inducers and inhibitors and GSH depletion studies indicate that both 2- and 3-methylacetaminophen are oxidized to reactive electrophilic metabolites, though further work is required to substantiate this hypothesis.

The analgesic potencies of the monomethylated analogues parallels their ability to cause hepatotoxicity. In one sense this is unfortunate, since we had hoped to find a nonhepatotoxic analogue that was active as an analgesic. On the other hand, this finding, if corroborated by further studies, may provide additional insight into the mechanism of analgesia produced by acetaminophen. Prostaglandin H synthase has been postulated as a target for the therapeutic activity of acetaminophen,[13] and both prostaglandin synthase and cytochrome P-450 catalyze the oxidation of the drug.[14] It is interesting that the oxidation potentials of the analogues studied here parallel both the analgesic activity and hepatotoxicity.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM 360A spectrometer at 60 MHz using tetramethylsilane as internal standard. Mass spectra were obtained on a VG 7070H high-resolution mass spectrometer at 70 eV. The peak intensities are given as a percentage of the base peak. Low-resolution spectra were determined at a nominal resolution $m/\Delta m$ = 1000 (10% valley), while high-resolution mass determinations were made at $m/\Delta m$ = 10 000. HPLC was performed on a Waters Associates (Milford, MA) liquid chromatograph equipped with a Model 440 absorbance detector, Model 6000A solvent delivery system, and U6K injector. Liquid scintillation counting of radioactive samples were carried out on a microprocessor-controlled Beckman LS-7500 system using a program that corrected for quench and background. Protein and GSH concentrations were determined on a Gilford Stasar II spectrophotometer. Centrifugations were performed either on a Sorvall Superspeed RC-2 refrigerated centrifuge or on a Beckman LS-65B refrigerated ultracentrifuge. Heparinized blood was centrifuged on a Beckman 152 Microfuge to obtain plasma samples. Incubation reactions were run in a LabLine/Dubnoff Incu-Shaker with water temperature set at 37 ± 0.5 °C and shaker set at 100 oscillations/min.

Electrochemical Measurements. Cyclic voltammetry was performed with a Bioanalytical Systems Model CV-27 cyclic voltammograph coupled to a C-1B cell stand. The voltammograms

(11) Hoffmann, K.-J.; Streeter, A. J.; Axworthy, D. B.; Baillie, T. A. *Mol. Pharmacol.* **1985**, *27*, 566.
(12) Emele, J. F.; Shanaman, J. *Proc. Soc. Exp. Biol. Med.* **1963**, *114*, 680.
(13) Flower, R. J.; Vane, J. R. *Nature (London)* **1972**, *240*, 410.
(14) (a) Mattammal, M. B.; Zenser, T. V.; Brown, W. W.; Herman, C. A.; Davis, B. B. *J. Pharmacol. Exp. Ther.* **1979**, *210*, 405. (b) Moldeus, P.; Rahimtula, A. *Biochem. Biophys. Res. Commun.* **1981**, *96*, 469. (c) Boyd, J. A.; Eling, T. E. *J. Pharmacol. Exp. Ther.* **1981**, *219*, 659.

were recorded on a Houston Instruments Model 100 X-Y recorder. The working electrode was a glassy carbon electrode that was polished with 0.05-μm polishing alumina between scans. An Ag/AgCl/3 M NaCl electrode was used as the reference. Scans were initiated at 0.0 V, in the positive direction at a scan rate of 150 mV/s. The concentrations of the compounds to be analyzed were 1 mM, and all samples were degassed with N_2 prior to running the scans. The samples were prepared in phosphate buffers of constant ionic strength (0.2 M).[15] The scans at ca. pH 0 were run in 1.8 M H_2SO_4.

Biological Experiments. Toxicology. Male Swiss-Webster mice (20 g) were obtained from Tyler Laboratories, Inc. (Bellevue, WA). Groups of 10 mice were injected ip with either acetaminophen or its monomethylated analogues at various dose levels in saline. Some groups were pretreated with sodium phenobarbital (80 mg/kg, ip) 72, 60, 48, and 24 h prior to the administration of drugs. Other groups received cobaltous chloride (30 mg/kg, ip) 48, 36, 24, and 12 h prior to experiments. Piperonyl butoxide was administered as a neat solution in a single 1360 mg/kg ip dose 30 min before experiments. Surviving animals were sacrificed by decapitation 24 h after receiving drugs. Blood was collected into heparinized beakers, pipetted into 1.0-mL disposable microcentrifuge tubes, and centrifuged for 3 min. Aliquots (200 μL) of plasma were transferred to clean microcentrifuge tubes and submitted to Pathologist's Central Laboratory (Seattle, WA) for ALT determinations. Liver and kidney sections were excised from surviving animals and fixed in buffered formalin. Paraffin sections were prepared and stained with hematoxylin and eosin, and the severity of necrosis was determined by Dr. Randy McMurtry, Pathologist, Presbyterian General Hospital (Denver, CO), by methods previously described for acetaminophen.[10] Relative hepatic glutathione levels were determined by the method of Ellman.[16]

Metabolism Studies. Groups of 12 mice were administered ip either [*acetyl*-^{14}C]-*N*-methylacetaminophen (3 mmol/kg, 0.45 mCi/mmol) or [*acetyl*-^{14}C]acetaminophen (3 mmol/kg, 0.45 mCi/mmol). Each animal was placed in a glass container fitted with a plastic screen for separation of urine and feces. Groups of 4 animals from each group were sacrificed at 1, 3, or 24 h by exsanguination into heparinized beakers. Various tissues, organs, and body fluids were immediately removed from each animal and placed into individual glass vials and frozen until workup. At each time point 0.5 g of liver was removed separately for determination of covalent binding as previously described for acetaminophen.[17] Approximately 100 mg of each tissue was weighed, minced, and digested in 2.0 mL of Soluene 350 (Packard Instrument Co., Downers Grove, IL). Upon complete dissolution (3–4 h) 10 mL of Dimilume scintillant was added, and the samples were counted by scintillation spectroscopy. Blood (0.2 mL) was digested in 1:1 Soluene 350/2-propanol (1.5 mL) and bleached with 0.5 mL of 30% hydrogen peroxide. Feces (20-mg samples) were processed by initial freeze-drying, followed by rehydration with 0.1 mL of water and dissolution in Soluene 350 (1 mL). After the samples were heated at 50 °C for 2 h, 0.5 mL of 2-propanol and 0.4 mL of 30% hydrogen peroxide were added to effect bleaching. Samples were then counted in 10 mL of Dimilume.

Urinary metabolites of acetaminophen were analyzed by HPLC using a previously published method[18] modified for collections of metabolites and counting of radioactivity. Urinary metabolites of *N*-methylacetaminophen were analyzed on a μBondapak C-18 reverse-phase column using a solvent system of H_2O/MeOH/HOAc (86.5:12.5:1 v/v) pumped at 2.0 mL/min. Under these conditions, metabolites eluted in the following order: 3-hydroxy-*N*-methylacetaminophen at 6.8 min, acetaminophen at 3.6 min, *N*-methylacetaminophen at 10.4 min, and 3-methoxy-*N*-methylacetaminophen at 14.0 min. Prior to analysis, samples of urine (0.5 mL) were hydrolyzed in 2.0 mL of 2 M acetate buffer, pH 5.0, containing 15000 EU of β-glucuronidase/sulfatase (Sigma Chemical Co., St. Louis, MO). Samples of liver homogenates from tissues taken 1 h after dosing were treated in a similar fashion to determine the nature of radiolabel present in this tissue.

Covalent binding was also measured in vitro using microsomes prepared from mouse liver. Reactions were carried out for 10 min at 37 °C using 2 mM concentrations of either radiolabeled acetaminophen or *N*-methylacetaminophen at the same specific activities as were used in the experiments in vivo. Covalent binding determinations were made as previously described for acetaminophen.[19]

Analgesic Testing. The bradykinin-induced writhing test for analgesia was determined for us by Hoffman-LaRoche Laboratories. Male CD1 mice weighing 15–20 g were used. Control groups (10 mice) received ip injections of 4 μg/0.2 mL of sterile water of bradykinin. After 15 min the mice received another 0.2 μg/0.1 mL injection of bradykinin, and writhing responses were monitored for the next 10 min. Other groups of mice received oral doses of test drugs 15 min prior to the first dose of bradykinin. Log probit analyses were carried out using at least five different doses of test drugs. Any drug having an ED_{50} value >200 mg/kg is considered inactive in this test.

Syntheses. 4′-Hydroxy-*N*-methylacetanilide (4). In a typical preparation, 4-(methylamino)phenol sulfate (2.0 g, 0.006 mol) was dissolved with heating in water (15 mL). After dissolution was complete, acetic anhydride (1.18 g, 0.012 mol) was added and immediately a white precipitate formed. The reaction was heated an additional 2 min and then allowed to cool to room temperature. The white solid was collected by suction filtration and recrystallized from $CH_3OH/CHCl_3$ (1:1) to give white prisms of 4′-hydroxy-*N*-methylacetanilide (*N*-methylacetaminophen, 4; 723 mg, 73%); mp 243–245 °C (lit.[20] mp 245 °C); ^{1}H NMR (Me_2SO-d_6) δ 1.90 (s, $COCH_3$), 3.10 (s, NCH_3), 3.60 (br s, OH), 6.75 (d, aromatic $H_{3'}$ and $H_{5'}$, J = 9 Hz), 7.10 (d, aromatic $H_{2'}$ and $H_{6'}$, J = 9 Hz).

4′-Hydroxy-*N*-[acetyl-1-^{14}*C*]-*N*-methylaniline ([*acetyl*-^{14}C]-*N*-Methylacetaminophen). This radiolabeled analogue of *N*-methylacetaminophen was prepared in an identical manner to the unlabeled compound. [1-^{14}C]Acetic anhydride (1.81 mg, 0.018 mmol, 55.3 mCi/mmol, Amersham, Arlington Heights, IL) was diluted with acetic anhydride (49 mg, 0.48 mmol) and added to a solution of 4-(methylamino)phenol sulfate (160 mg, 0.46 mmol) in 2.0 mL of H_2O. After recrystallization the product was obtained as white plates (51 mg, 67% yield). Radioactive purity was found to be approximately 98% by HPLC using the conditions described for metabolism studies (sp. act. 1.35 mCi/mmol).

4′-Hydroxy-*N*-[acetyl-1-^{14}*C*]aniline ([*acetyl*-^{14}C]Acetaminophen). [1-^{14}C]Acetic anhydride (59 mg, 0.58 mmol, 2.98 mCi/mmol, ICN Chemical and Radioisotope Division, Irvine, CA), was diluted with acetic anhydride (165 mg, 1.62 mmol) and added to a solution of 4-aminophenol (218 mg, 2.0 mmol) in sodium acetate buffer, pH 5.0 (3 mL) and rapidly stirred for 1 h with heating to 60 °C. The reaction mixture was cooled and crystalline solid collected by suction filtration. The solid was recrystallized from water 2 times to yield small, off-white cubic crystals (252 mg, 84% yield). Radioactive purity was found to be 98.4% by HPLC using the conditions described for metabolism studies (sp. act. 0.45 mCi/mmol).

4′-Hydroxy-2′-methylacetanilide (5). Due to poor solubility, 4-hydroxy-2-methylaniline (10.0 g, 0.08 mol) was first dissolved in a solution of HCl (7 mL of concentrated HCl/135 mL of H_2O) followed by the rapid addition of sodium acetate (11.0 g/30 mL of H_2O) and acetic anhydride (11.0 g, 0.108 mol). The solution was stirred at room temperature for 45 min. Cooling in an ice bath yielded a solid that was collected by suction filtration and recrystallized from hot H_2O. The desired product, 2-methylacetaminophen (5.8 g, 43.5% yield), had mp 128–129 °C (lit.[9a] mp 130 °C); ^{1}H NMR (acetone-d_6) δ 2.00 (s, $COCH_3$), 2.10 (s, 2′-CH_3), 6.50 (d, aromatic $H_{5'}$, J = 8 Hz), 6.58 (s, aromatic $H_{3'}$), 7.10 (d, aromatic $H_{6'}$, J = 8 Hz).

(15) Christian, G. D. *Analytical Chemistry*, 3rd ed.; Wiley: New York, 1980; p 186.
(16) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.
(17) Jollow, D. J.; Mitchell, J. R.; Potter, W. Z.; Davis, D. C.; Gillette, J. R.; Brodie, B. B. *J. Pharmacol. Exp. Ther.* **1973**, *187*, 195.
(18) Wilson, J. M.; Slattery, J. T.; Forte, A. J.; Nelson, S. D. *J. Chromatogr.* **1982**, *227*, 453.
(19) Potter, W. Z.; Davis, D. C.; Mitchell, J. R.; Jollow, D. J.; Gillette, J. R.; Brodie, B. B. *J. Pharmacol. Exp. Ther.* **1973**, *187*, 203.
(20) Meldola, R.; Hollely, W. F. *J. Chem. Soc.* **1914**, *105*, 2073.

4′-Hydroxy-3′-methylacetanilide (6). Sulfanilic acid (17.3 g, 0.09 mol) and sodium carbonate (5.3 g, 0.05 mole) were dissolved in 100 mL of H_2O with heating and stirring. This was cooled to 15 °C; a mixture of sodium nitrite (7.4 g, 0.107 mol) in H_2O (20 mL) was added over a 1-min period, and the entire mixture was then poured onto 120 g of crushed ice containing concentrated HCl (21 mL). The diazotized sulfanilic acid was subsequently added with stirring to a cooled solution of *o*-cresol (10.8 g, 0.1 mol in 120 mL of 0.3 M NaOH), and the mixture was stirred for 3 h at 5 °C. This mixture was then heated to 45–50 °C, and sodium dithionite (42.0 g, 0.20 mol) was added in small portions so that the temperature was maintained at 60–65 °C.

The 4-hydroxy-3-methylaniline that precipitated was washed with cold water and dissolved in glacial acetic acid (15 mL), and acetic anhydride (12.8 g, 0.13 mol) was added while stirring over 10 min. 3-Methylacetaminophen was collected by suction filtration and recrystallized from 95% EtOH to give fine white needles (6.92 g, 41% yield): mp 179–180 °C (lit.[9b] mp 179 °C); ^{1}H NMR (methanol-d_4) δ 2.10 (s, $COCH_3$), 2.20 (s, 3′-CH_3), 6.60 (d, aromatic $H_{5'}$, J = 8 Hz), 7.10 (d, aromatic $H_{6'}$, J = 8 Hz), 7.15 (s, aromatic $H_{2'}$).

Spiro[1,3-benzodioxole-2,1′-cyclohexane] (7). A solution of catechol (6.67 g, 0.06 mol), cyclohexanone (5.88 g, 0.06 mol), and *p*-toluenesulfonic acid (catalytic amount, ca. 2 mg) was refluxed in toluene (60 mL) for 24 h. The water generated by the reaction (ca. 1 mL, 92.5% theoretical yield) was collected in a Dean-Stark trap. The reaction solution was subsequently extracted with 5% aqueous NaOH solution (3 × 60 mL), followed by washing with H_2O (2 × 10 mL). After the organic layer was dried over Na_2SO_4, it was concentrated under reduced pressure to a brown oil that solidified on standing. Recrystallization from petroleum ether (charcoal) afforded 4.13 g (36.2% yield) of **7** as colorless plates: mp 47.5–49 °C (lit.[21] mp 46–48 °C); ^{1}H NMR ($CDCl_3$) δ 1.3–2.2 (br m, 10 H, cyclohexylidene CH_2), 6.77 (s, 4 H, aromatic H).

5-Nitrospiro[1,3-benzodioxole-2,1′-cyclohexane] (8). To 6 mL of 60% aqueous HNO_3 at 0 °C was added a solution of **7** (958.8 mg, 5.0 mmol) in EtOH (6 mL) dropwise over a 15-min period. The solution immediately turned yellow followed by the rapid formation of a yellow solid. The crude product was collected, washed with H_2O, and recrystallized from EtOH to yield 949.3 mg (80.7%) of **8** as fine, yellow needles: mp 99.5–101.5 °C (lit.[22] mp 99–100 °C); ^{1}H NMR ($CDCl_3$) δ 1.3–2.3 (br m, 10 H, cyclohexylidene CH_2), 6.77 (d, 1 H, $J_{7,6}$ = 9 Hz, aromatic H_7), 7.59 (s, 1 H, $J_{4,6}$ = 2 Hz, aromatic H_4), 7.86 (dd, H_4), H, $J_{6,4}$ = 2 Hz, $J_{6,7}$ = 9 Hz, aromatic H_6).

***N*-Acetyl-5-aminospiro[1,3-benzodioxole-2,1′-cyclohexane] (9).** A solution of **8** (0.94 g, 4.0 mmol) in MeOH (60 mL) was hydrogenated at 3 atm over 10% Pd–C (55 mg) for 3.5 h. The reaction mixture was then filtered through Celite; acetic anhydride (0.61 g, 6.0 mmol) was added, and the solution was stirred at room temperature. After 1 h the solvent was removed under reduced pressure yielding a brown oil. This residue was chromatographed on silica gel 60 (40 g, 70–230 mesh) eluting successively with $CHCl_3$ (300 mL) and $CHCl_3$/MeOH (95:5, 300 mL). The appropriate fractions were combined and evaporated to a brown oil that slowly solidified. Recrystallization of the crude product from $CHCl_3$/hexane gave 690.3 mg (69.8% yield) of **9**: mp 94.5–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.2–2.2 (br m, 10 H, cyclohexylidene CH_2), 2.10 (s, 3 H, $COCH_3$), 6.63 (br s, 2 H, aromatic H), 7.07 (s, 1 H, aromatic H), 7.70 (br m, 1 H, NH); IR (KBr) ν 2926 (m, CH_2), 1651 (s, C=O), 1492 (s, CH_2) cm^{-1}; UV (MeOH) λ_{max} 259 nm (ϵ 7700), 297 (5000); EIMS, *m/e* 247 (M^+), 205 (M – CH_2CO), 204 (M – CH_3CO), 43 (CH_3CO^+). Calcd for $C_{14}H_{17}NO_3$, 247.1208. Found, 247.1218.

***N*-Acetyl-*N*-methyl-5-aminospiro[1,3-benzodioxole-2,1′-cyclohexane] (10).** This compound was prepared by modification of a previously described method.[23] To a stirred solution of **9** (503.6 mg, 2.04 mmol) in dry Me_2SO (2 mL) was added pulverized KOH (574.7 mg, 10.24 mmol) in one portion. The reaction mixture immediately turned dark brown with some gas evolution. A solution of CH_3I (1.46 g, 10.28 mmol) in Me_2SO (2 mL) was then added dropwise over a 5-min period. After stirring at room temperature for 2 h, the reaction was quenched with H_2O (20 mL) and extracted with Et_2O (3 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to a yellow oil, which solidified in the freezer. Recrystallization of the crude product from hexane gave 150.8 mg (28.3% yield) of **10**: mp 149–151 °C; ^{1}H NMR ($CDCl_3$) δ 1.87 (br m, 13 H, overlap of cyclohexylidene CH_2 and $COCH_3$), 3.17 (s, 3 H, NCH_3), 6.60 (m, 3 H, aromatic H); IR (KBr) ν 2936 (m, CH_2), 1657 (s, C=O), 1496 (s, CH_2) cm^{-1}; UV (MeOH) λ_{max} 244 nm (ϵ 4800), 290 (5000); EIMS, *m/e* 261 (M^+), 219 (M – CH_2CO), 218 (M – CH_3CO), 43 (CH_3CO^+). Calcd for $C_{15}H_{19}NO_3$, 261.1365. Found, 261.1374.

3′,4′-Dihydroxy-*N*-methylacetanilide (4a). The synthesis followed the method of Boechmann and Schill.[24] A solution of **10** (134.8 mg, 0.52 mmol) in EtOH (12.5 mL) and concentrated HCl (3.0 mL) was heated at gentle reflux under N_2 for 90 min. The solution was then concentrated to a brown oil in vacuo, dissolved in H_2O (10 mL), and extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated to a brown oil that solidified on standing in the freezer. Recrystallization of the crude product from MeOH provided 28.7 mg (30.5% yield) of **4a** as a white solid: mp 153–154.5 °C; ^{1}H NMR (CH_3OH-d_4) δ 1.84 (s, 3 H, $COCH_3$), 3.17 (s, 3 H, NCH_3), 6.57 (dd, 1 H, $J_{6',2'}$ = 2.58 Hz, $J_{6',5'}$ = 8.60 Hz, aromatic $H_{6'}$), 6.65 (d, 1 H, $J_{2',6'}$ = 2.58 Hz, aromatic $H_{2'}$), 6.80 (d, 1 H, aromatic $H_{5'}$); IR (KBr) ν 2500–3500 (s, OH), 1601 (s, C=O), 1515 (m), 1470 (s), 1386 (s), 1305 (s), 1280 (s), 1210 (s) cm^{-1}; UV (MeOH) λ_{max} 235 nm (ϵ 7700), 283 (3100); EIMS, *m/e* 181 (M^+), 139 (M – CH_2CO), 138 (M – CH_3CO), 43 (CH_3CO^+). Calcd for $C_9H_{11}NO_3$, 181.0736. Found, 181.0742.

3-Methyl-4-hydroxynitrobenzene (11). A mixture of 3,4-dimethoxynitrobenzene (5.00 g, 0.027 mol) in 10% aqueous KOH solution (100 mL) was heated at reflux for 24 h. Upon cooling a reddish orange precipitate formed that was dissolved in H_2O (400 mL). Addition of concentrated HCl (25 mL) resulted in discharge of the red color and formation of a feathery yellow solid. The product was collected, washed with H_2O, and dried under vacuum (45° C/P_2O_5) to afford 3.79 g (83.0% yield) of **11** as a yellow solid: mp 99.5–101.5 °C (lit.[25] mp 101 °C); ^{1}H NMR ($CDCl_3$) δ 4.00 (s, 3 H, OCH_3), 6.30 (s, 1 H, OH), 6.97 (s, 1 H, $J_{5,6}$ = 8 Hz, aromatic H_5), 7.78 (br s, 1 H, aromatic H_2), 7.96 (d, 1 H, $J_{6,2}$ = 2 Hz, aromatic H_6).

3-Methoxy-4-(α-tetrahydropyranyloxy)nitrobenzene (12). A standard method for formation of tetrahydropyranyl ethers was used.[26] To a stirred solution of **11** (1.02 g, 6.0 mmol) in EtOAc (10 mL) was added HCl-saturated EtOAc (0.5 mL), followed by dihydropyran (4.34 g, 51.6 mmol). After stirring at room temperature for 18 h, the reaction solution was extracted with 10% aqueous NaOH solution (5 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to a yellow oil that slowly solidified on standing. Recrystallization from EtOH yielded 0.79 g (52.1%) of **12** as fine, yellow needles: mp 84.5–87 °C; ^{1}H NMR ($CDCl_3$) δ 1.77 (br m, 6 H, tetrahydropyranyl $CH_2CH_2CH_2$), 3.77 (br m, 2 H, OCH_2), 3.93 (s, 3 H, OCH_3), 5.53 (br m, 1 H, OCH), 7.17 (d, 1 H, $J_{5,6}$ = 9 Hz, aromatic H_5), 7.75 and 7.92 (br s and d, 2 H, overlapping aromatic H_2 and H_6); IR (KBr) ν 2929 (s, CH_2), 1584 (s), 1504 (s, NO_2), 1339 (s, NO_2), 1268 (s, C–O–C), 872 (s, NO_2) cm^{-1}; UV (MeOH) λ_{max} 237 nm (ϵ 9600), 298 (6600), 334 (7500); CIMS (CH_4), *m/e* 170 (MH^+ – C_5H_9O), 85 ($C_5H_8OH^+$).

3′-Methoxy-4′-(α-tetrahydropyranyloxy)acetanilide (13). A solution of **12** (627.2 mg, 2.50 mmol) and acetic anhydride (0.52 g, 5.10 mmol) in MeOH (40 mL) was hydrogenated at 3 atm over 10% Pd–C (37.2 mg). After 3 h the reaction mixture was filtered through Celite and concentrated to a brown oil under reduced pressure. The rotovap residue was dissolved in EtOAc (50 mL) and extracted with 5% aqueous NaOH solution (3 × 25 mL),

(21) Cole, E. R.; Crank, G.; Hai Minh, H. T. *Aust. J. Chem.* **1980**, *33*, 675.
(22) Clark, R. L.; Pessolano, A. A.; Shen, T.-Y.; Jacobus, D. P.; Jones, H.; Lotti, V. J.; Flataker, L. M. *J. Med. Chem.* **1978**, *21*, 965.
(23) Isele, G. L.; Luttringhaus, A. *Synthesis* **1971**, 266.
(24) Boechmann, J.; Schill, G. *Chem. Ber.* **1977**, *110*, 703.
(25) Pollecoff, F.; Robinson, R. *J. Chem. Soc.* **1918**, *113*, 645.
(26) Grant, H. N.; Prelog, V.; Sneeden, R. P. A. *Helv. Chim. Acta* **1963**, *46*, 415.

followed by H_2O (1 × 25 mL). After drying over $MgSO_4$ the organic layer was evaporated to a tan solid. The crude product was recrystallized from MeOH to yield 317.0 mg (47.8%) of **13**: mp 118–121 °C; ^1H NMR ($CDCl_3$) δ 1.80 (br m, 6 H, tetrahydropyranyl $CH_2CH_2CH_2$), 2.10 (s, 3 H, $COCH_3$), 3.78 (overlapping m and s, 5 H, OCH_3 and THP–OCH_2), 5.28 (br s, 1 H, THP–OCH), 6.73 (dd, 1 H, $J_{6',2'}$ = 2 Hz, $J_{6',5'}$ = 9 Hz, aromatic $H_{6'}$), 7.02 (d, 1 H, $J_{5',6'}$ = 9 Hz, aromatic $H_{5'}$), 7.35 (d, 1 H, $J_{2',6'}$ = 2 Hz, aromatic $H_{2'}$), 7.70 (br s, 1 H, NH); IR (KBr) ν 3236 (m), 2928 (s, CH_2), 1648 (s, C=O), 1468 (m, CH_2), 1233 (s, C–O–C) cm^{-1}; UV (MeOH) λ_{max} 251 nm (ε 13600), 286 (4200); CIMS (CH_4), *m/e* 266 (MH^+), 182 ($C_9H_{11}NO_3$), 181 ($C_9H_{11}NO_3$) *or* ($MH^+ - C_5H_8O$), 139 ($C_9H_{11}NO_3$–CH_2CO), 85 ($C_5H_8OH^+$), 43 (CH_3CO^+).

3′-Methoxy-4′-(α-tetrahydropyranyloxy)-*N*-methylacetanilide (14). To a stirred solution of **13** (202.5 mg, 0.76 mmol) in dry Me_2SO (1 mL) was added pulverized KOH (174.0 mg, 3.10 mmol). The reaction mixture was stirred at room temperature for 5 min at which point a solution of CH_3I (0.43 g, 3.05 mmol) in Me_2SO (2 mL) was added over a 2-min period. After stirring for 90 min the reaction mixture was quenched with H_2O (10 mL) and then extracted with Et_2O (5 × 10 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated to a white solid. Recrystallization from MeOH gave 72.9 mg (34.3% yield) of 14 as a white solid: mp 109–111 °C; ^1H NMR ($CDCl_3$) δ 1.80 (br m, 9 H, overlap of $COCH_3$ and tetrahydropyranyl $CH_2CH_2CH_2$), 3.17 (s, 3 H, NCH_3), 3.80 (m, 5 H, OCH_3 and THP–OCH_2), 5.33 (m, 1 H, THP–OCH), 6.67 (m, 2 H, aromatic H), 7.20 (m, 1 H, aromatic H); IR (KBr) ν 2945 (m, CH_2), 1650 (s, C=O), 1245 (s, C–O–C) cm^{-1}; UV (MeOH) λ_{max} 233 nm (ε 9400), 280 (2900); CIMS (CH_4), *m/e* 280 (MH^+), 196 ($C_{10}H_{13}NO_3$ + H), 195 ($MH^+ - C_5H_3O$), 85 ($C_5H_8OH^+$), 43 (CH_3CO^+).

3′-Methoxy-4′-hydroxy-*N*-methylacetanilide (4b). The THP ether was removed by the method of Beier and Mundy.[27] A mixture of **14** (212.3 mg, 0.76 mmol) and Dowex 50W (H^+) resin (325.0 mg) were stirred together in MeOH (2 mL) for 1 h at room temperature. The reaction mixture was then filtered and evaporated to a yellow solid under reduced pressure. The crude product was recrystallized twice from MeOH (charcoal) to yield 19.2 mg (13.2%) of **4b** as a white crystalline solid: mp 135–136 °C; ^1H NMR (CH_3OH-d_4) δ 1.87 (s, 3 H, $COCH_3$), 3.22 (s, 3 H, NCH_3), 3.88 (s, 3 H, OCH_3), 6.68 (dd, 1 H, $J_{6',2'}$ = 2.58 Hz, $J_{6',5'}$ = 7.74 Hz, aromatic $H_{6'}$), 6.78 (d, 1 H, $J_{2',6'}$ = 2.58 Hz, aromatic $H_{2'}$), 6.85 (d, 1 H, $J_{5',6'}$ = 8.59 Hz, aromatic $H_{5'}$); IR (KBr) ν 2500–3500 (m, OH), 1619 (s, C=O), 1601 (s), 1529 (s), 1459 (s), 1323 (m), 1284 (s), 1249 (s), 1220 (s), 1175 (s), 1129 (m) cm^{-1}; UV (MeOH) λ_{max} 235 nm (ε 8000), 283 (3200); EIMS, *m/e* 195 (M^+), 153 (M – CH_2CO), 152 (M – CH_3CO), 138 (M – CH_2CO – CH_3), 137 (M – CH_3CO – CH_3), 43 (CH_3CO^+). Calcd for $C_{10}H_{13}NO_3$, 195.0892. Found, 195.0891.

Registry No. 4, 29121-34-4; 4 (1-^{14}C), 102683-54-5; **4A**, 54826-77-6; **4B**, 102683-60-3; **5**, 39495-15-3; **6**, 16375-90-9; **7**, 182-55-8; **8**, 64179-40-4; **9**, 102683-55-6; **10**, 102683-56-7; 11, 3251-56-7; **12**, 102683-57-8; **13**, 102683-58-9; **14**, 102683-59-0; 4-$H_3CNHC_6H_4OH{\cdot}H_2SO_4$, 55-55-0; 4-$HOC_6H_4NH^{14}COCH_3$, 60218-46-4; 4-$H_2NC_6H_4OH$, 123-30-8; 4-$H_2NC_6H_4SO_3H$, 121-57-3; 4-$^+N_2C_6H_4SO_3H{\cdot}Cl^-$, 6118-33-8; 2-$HOC_6H_4CH_3$, 95-48-7; 4-hydroxy-2-methylaniline, 2835-99-6; 4-hydroxy-3-methylaniline, 2835-96-3; catechol, 120-80-9; cyclohexanone, 108-94-1; 3,4-dimethoxynitrobenzene, 709-09-1.

(27) Beier, R.; Mundy, B. P. *Synth. Commun.* **1979**, *9*, 271.

Isozyme-Specific Enzyme Inhibitors. 12.[1] *C*- and *N*-Methylmethionines as Substrates and Inhibitors of Methionine Adenosyltransferases of Normal and Hepatoma Rat Tissues

Hong Lim, Francis Kappler, Ton T. Hai, and Alexander Hampton*

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received October 15, 1985*

The 2-, 3-, and 4-mono-*C*-methyl derivatives of D,L-methionine (Met) have been resolved into the 10 possible enantiomeric forms having the configurations 2-Me-D, 2-Me-L, 3(α or β)-Me-D, 3(α or β)-Me-L, 4(α or β)-Me-D, and 4(α or β)-Me-L (the α designation was given to enantiomeric pairs that had higher R_f values on silica gel chromatograms than their diastereomeric counterparts). All compounds were weak, poorly selective inhibitors of the rat M-2 (normal tissue) and M-T (Novikoff ascitic hepatoma) variants of Met adenosyltransferase. Kinetic analysis of the three most effective showed them to be competitive inhibitors with respect to Met with both variants; the strongest inhibition (K_M(Met)/K_i = 0.03) was that of M-T by 3β-Me-L-Met. The Me-Met enantiomers had low substrate efficiencies (V_{max}/K_M) in the range (0.5–2.2) × 10^{-4} that of L-Met with M-2 and (0.2–1.3) × 10^{-3} with M-T among seven compounds studied. At a 4 mM level, seven of the enantiomers were converted to adenosylmethionine derivatives more rapidly by M-T than by M-2. Among these, 2-Me-L-Met, 3α-Me-L-Met, 3α-Me-D-Met, and 4β-Me-D-Met had little or no substrate activity with M-2. These differences in substrate specificity are potentially exploitable in the design of compounds with selective toxicity for rat tumor tissue. *N*-Me- and *N*-(*n*-Bu)-Met, and the Met analogue in which NH is substituted for S, were weak inhibitors of M-T and M-2 and showed no substrate activity at a level of 4 mM.

The isozyme composition of mammalian normal and neoplastic tissues indicates, on grounds discussed previously,[2] that isozyme-specific inhibitors are potentially useful as progenitors of new antineoplastic agents. In previous work in this series, we have studied several approaches to the design of such inhibitors. These studies were carried out with four enzymes, each of which occurs as a variant that is of interest as a possible target in cancer chemotherapy. It was found that monosubstituted substrate derivatives that bear short substituents at various positions frequently possess substrate or inhibitor properties that are isozyme selective.[3] In addition, it was found that such derivatives can sometimes serve as starting points in the development of compounds with enhanced selectivity and/or inhibitory potency.[4–6] Recently,[3] we described isozyme-selective inhibitory effects produced by monosubstituted adenosine 5′-triphosphate (ATP) deriv-

(1) For part 11 of this series see: Kappler, F.; Hai, T. T.; Cotter, R. J.; Hyver, K. J.; Hampton, A. *J. Med. Chem.* **1986**, *29*, 1030.
(2) Hampton, A.; Kappler, F.; Maeda, M.; Patel, A. D. *J. Med. Chem.* **1978**, *21*, 1137.
(3) Kappler, F.; Hai, T. T.; Hampton, A. *J. Med. Chem.*, in press.
(4) Hampton, A.; Kappler, F.; Picker, D. *J. Med. Chem.* **1982**, *25*, 638.
(5) Hampton, A.; Chawla, R. R.; Kappler, F. *J. Med. Chem.* **1982**, *25*, 644.
(6) Kappler, F.; Hai, T. T.; Abo, M.; Hampton, A. *J. Med. Chem.* **1982**, *25*, 1179.

0022-2623/86/1829-1743$01.50/0