Journal of Medicinal Chemistry

Volume 23, Number 11 November 1980

Articles

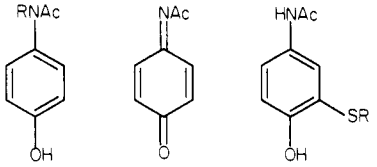
Studies on the Mechanism of Toxicity of Acetaminophen. Synthesis and Reactions of *N*-Acetyl-2,6-dimethyl- and *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imines

C. Roger Fernando, Ian C. Calder,* and Kathryn N. Ham

Departments of Chemistry and Pathology, University of Melbourne, Parkville, Victoria, Australia 3052.
Received December 3, 1979

N-Acetyl-2,6-dimethyl-*p*-benzoquinone imine and *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine were prepared from 2,6-dimethylacetaminophen and 3,5-dimethylacetaminophen by oxidation with lead tetraacetate. Reaction of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine with hydrochloric acid gave 3′-chloro-2′,6′-dimethyl-4′-hydroxyacetanilide, whereas ethanethiol, aniline, and ethanol gave tetrahedral adducts resulting from addition to the imine carbon. Water gave 2,6-dimethyl-*p*-benzoquinone. With *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine, water and aniline gave substitution on the imine carbon, yielding 2,6-dimethyl-*p*-benzoquinone and 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine, respectively. Ethanethiol gave 3′,5′-dimethyl-2′-(ethylthio)-4′-hydroxyacetanilide. The toxicity of 2,6-dimethylacetaminophen and 3,5-dimethylacetaminophen was examined histologically in mice and rats. 3,5-Dimethylacetaminophen was slightly more nephrotoxic but showed a similar hepatotoxicity to acetaminophen. 2,6-Dimethylacetaminophen, like *N*-methylacetaminophen, showed very little tissue damage.

The hepatotoxicity and nephrotoxicity of acetaminophen (**1**)[2] in man and experimental animals have been attributed



1, R = H
2, R = OH
5, R = CH_3

3 **4**

to metabolic conversion of the drug into a reactive intermediate by the cytochrome P_{450} dependent mixed function oxidase system.[1] *N*-Acetyl-*p*-benzoquinone imine (**3**)[2] is the most likely structure for the ultimate reactive intermediate. Whether metabolic oxidation of acetaminophen results in the formation of the quinone imine directly or via *N*-hydroxyacetaminophen (**2**) has not yet been resolved.[3] The properties[4-6] of *N*-hydroxyacetaminophen, together with the failure[3] of hamster liver microsomes to form *N*-hydroxyacetaminophen, suggest that the toxic reactive intermediate does not arise through the formation of *N*-hydroxyacetaminophen.

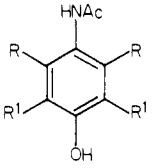
With therapeutic doses, a large proportion of the acetaminophen is excreted as the glucuronide and sulfate conjugates and most of the *N*-acetyl-*p*-benzoquinone imine formed reacts with glutathione to ultimately become water soluble, nontoxic metabolites (**4**) which are excreted.[7] When a larger dose is administered, the level of glutathione is depleted and the quinone imine reacts with cell macromolecules leading to cell damage or death. The reaction with cell macromolecules is associated with covalent binding, so that the amount of binding to cellular protein correlates well with the extent of necrosis observed,[8] although there are cases where cell death has been shown not to occur under circumstances where covalent binding is already maximal.[9,10] The mechanism of acetaminophen-induced toxicity has come under extensive study and circumstantial evidence for the involvement of the cytochrome P_{450} mixed function oxidase in the formation of an electrophilic reactive intermediate is strong. The toxicity observed in mice is enhanced by inducers of drug metabolism and reduced by inhibitors, demonstrating an oxidation step by the cytochrome P_{450} dependent mixed function oxidase system.[11] The introduction of a methyl

(1) J. R. Mitchell, R. J. McMurtry, C. N. Stratham, and S. D. Nelson, *Am. J. Med.*, **62**, 518 (1977).
(2) Systematic nomenclature: acetaminophen = 4′-hydroxyacetanilide; *N*-acetyl-*p*-benzoquinone imine = 4-(acetylimino)-2,5-cyclohexadien-1-one.
(3) J. A. Hinson, L. R. Pohl, and J. R. Gillette, *Life Sci.*, **24**, 2133 (1979).
(4) K. Healey, I. C. Calder, A. C. Yong, C. A. Crowe, C. C. Funder, K. N. Ham, and J. D. Tange, *Xenobiotica*, **8**, 403 (1978).
(5) K. Healey and I. C. Calder, *Aust. J. Chem.*, **32**, 1307 (1979).
(6) M. W. Gemborys, G. W. Gribble, and G. H. Mudge, *J. Med. Chem.*, **21**, 649 (1978).
(7) D. J. Jollow, S. S. Thorgeirsson, W. Z. Potter, M. Hashimoto, and J. R. Mitchell, *Pharmacology*, **12**, 251 (1974).
(8) M. Davis, N. G. Harrison, G. Ideo, B. Portmann, D. Labadarios. and R. William, *Xenobiotica*, **6**, 249 (1976).
(9) D. Labadarios, M. Davis, B. Portmann, and R. Williams, *Biochem. Pharmacol.*, **26**, 31 (1977).
(10) J. G. Gerber, J. S. MacDonald, R. D. Harbison, J. Villeneuve, A. J. J. Wwod, and A. S. Neis, *Lancet*, 1, 657 (1977).

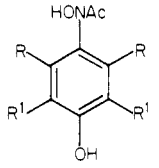
0022-2623/80/1823-1153$01.00/0

group on the nitrogen atom substantially reduces the toxicity, suggesting that oxidation occurs at the nitrogen atom.[12] The results of studies with ^{18}O are also consistent with an oxidation step which retains the original oxygen atom of the hydroxyl group.[13] Further, under conditions where the glutathione levels are reduced, a marked increase in toxicity is observed.[14] The present treatment of acetaminophen overdosage aims at restoring the glutathione levels or providing alternative nucleophiles by administering methionine or cysteine.[15] With most compounds the cytochrome P_{450} dependent mixed function oxidase system carries out an oxidation of the substrate by the introduction of an oxygen atom into the molecule. The oxidation of acetaminophen directly to *N*-acetyl-*p*-benzoquinone imine would represent an unusual mode of action for this drug-metabolizing system.

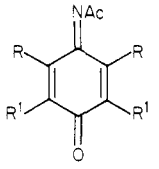
To further elucidate the mechanisms involved in the metabolic activation and toxicity of acetaminophen, we have undertaken a continuing study of the chemistry and toxicity of the ring methyl substituted acetaminophen derivatives. The substitution of methyl groups on the benzene ring of the acetaminophen would be expected to influence the reactivity of both the acetaminophen toward metabolic reactions and the reactions of possible toxic reactive intermediates. In this paper we present a preliminary report of the toxicity of 3,5-dimethylacetaminophen (**6**) and 2,6-dimethylacetaminophen (**7**), compounds



6, R = H; R^1 = Me
7, R = Me; R^1 = H



8, R = H; R^1 = Me
9, R = Me; R^1 = H



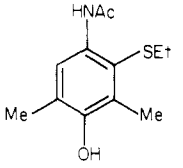
10, R = H; R^1 = Me
11, R = Me; R^1 = H

in which the methyl groups are symmetrically substituted. As well, an investigation of the chemistry of *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**) and *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (**11**) is described. These are the reactive electrophilic intermediates which would be expected if the dimethylacetaminophens **6** and **7** were metabolized in the same way as acetaminophen.

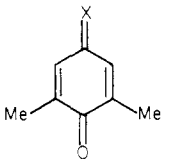
Chemistry. 2,6-Dimethyl-4-hydroxyaniline was prepared by coupling 3,5-dimethylphenol with diazotized sulfanilic acid followed by reduction[16] of the newly formed azo dye, and 3,5-dimethyl-4-hydroxyaniline was prepared by nitrosation of 2,6-dimethylphenol followed by catalytic reduction. Acetylation of the 4-hydroxyanilines with acetic anhydride gave the substituted acetaminophens in good yield. *N*-Methylacetaminophen was prepared by acetylating a commercial sample of *N*-methyl-4-hydroxyaniline. Oxidation of the dimethylacetaminophens **6** and **7** with $Pb(OAc)_4$ in ethyl acetate yielded the corresponding *N*-acetyldimethyl-*p*-benzoquinone imines **10** and **11** as pale yellow crystalline compounds, which were recrystallized under very mild conditions. The ^{13}C spectra of the quinone imines **10** and **11** showed the carbonyl carbons at δ 170.6 and 186.4 and the imine carbons at δ 143.7 and 150.9, respectively. The carbonyl carbons are similar to those observed[17] for 2,6-dimethyl-*p*-benzoquinone, δ 187.6 and 188.3, which are distinctly different from the corresponding carbons in the acetaminophens which are observed at δ 149.0 and 155.6 next to the hydroxyl and δ 131.1 and 136.43 next to the acetamido groups, respectively. Reduction of the dimethylquinone imines **10** and **11** with dithionite gave the corresponding acetaminophens **6** and **7** in high yields.

Comparison of the chemical shifts together with the general symmetry of the spectra easily allowed characterization of the substituted compounds prepared. All substituted compounds showed the expected mass spectra and infrared spectra.

The dimethylquinone imines were allowed to react with a number of nucleophiles containing Cl, S, N, or O as the nucleophilic hetero atom. With *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**), ethanethiol reacted smoothly to yield 3′,5′-dimethyl-2′-(ethylthio)-4′-hydroxyacetanilide (**12**). However with hydrochloric acid only polymeric



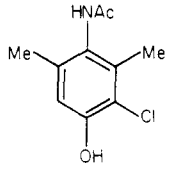
12



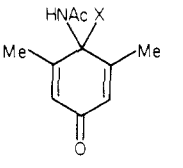
13, X = O
14, X = NC_6H_5

material could be isolated. With water and aniline, reaction occurred at the imine carbon followed by loss of acetamide to give 2,6-dimethyl-*p*-benzoquinone (**13**) and 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine (**14**), respectively. The *N*-phenyl-*p*-benzoquinone imine (**14**) was identical with a sample prepared by coupling nitrosobenzene with 2,6-dimethylphenol as described by Ried and Neidhardt.[18]

Reaction of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (**11**) with hydrochloric acid gave 3′-chloro-2′,6′-dimethyl-4′-hydroxyacetanilide (**15**), whereas the other nucleophiles



15



16, X = SEt
17, X = NHC_6H_5
18, X = OEt

gave products resulting from addition to the imine carbon. With water the addition is followed by loss of acetamide to give 2,6-dimethyl-*p*-benzoquinone (**13**). When ethanethiol, aniline, and ethanol were added to the 2,6-di-

(11) W. Z. Potter, D. D. Davis, J. R. Mitchell, D. J. Jollow, J. R. Gillette, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **187**, 203 (1973).
(12) S. D. Nelson, A. J. Forte, and R. J. McMurtry, *Res. Commun. Chem. Pathol. Pharmacol.*, **22**, 61 (1978).
(13) J. A. Hinson, S. D. Nelson, and J. R. Gillette, *Mol. Pharmacol.*, **15**, 419 (1979).
(14) J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **187**, 211 (1973).
(15) See Symposium on Paracetamol and the Liver, *J. Int. Med. Res.*, 4 (Suppl 4) (1976).
(16) L. F. Fieser, "Organic Syntheses", Collect. Vol. II, Wiley, New York, 1943, p 39.
(17) St. Berger and A Rieker, *Tetrahedron*, **28**, 3123 (1972).
(18) W. Ried and H. Neidhardt, *Chem. Ber.*, **94**, 373 (1961).

Table I. Nephrotoxicity and Hepatotoxicity of Acetaminophen and Methyl-Substituted Derivatives in Female Rats and Mice[a]

	rats						mice			
	iv[b]		ip[c]		ig[d]		ip[c]		ig[d]	
	kidney	liver	kidney	liver	kidney	liver	kidney	liver	kidney	liver
acetaminophen (1)										
1 mM	0	0	0	0			0	++		
2 mM	0	0	0	+	0	0	0	+++	0	++
5 mM					0	+			0	+++
10 mM					+	+++			0	++++
N-methylacetaminophen (5)										
1 mM			0	0			0	0		
2 mM			0	0			0	0		
3,5-dimethylacetaminophen (7)										
1 mM	0	+								
2 mM	+	++			0	+			+	++
5 mM					+	++			+	+++
10 mM					+	++			+	++++
2,6-dimethylacetaminophen (6)										
2 mM	0	0			0	0			0	0
5 mM					0	0			0	0
10 mM					0	+			0	0

[a] Graded histologically 48 h after administration; at least five animals were used at each dose level for each route of administration. Toxicity was assessed (0 = normal; ++++ = most severe) in kidney as proximal tublar necrosis deep in the cortex, and in the liver as centrilobular vacuolation or necrosis. [b] Intravenous. [c] Intraperitoneal. [d] Intragastric.

methyl-p-benzoquinone imine (11), the products were the tetrahedral adducts 16–18 in which the acetamido group was retained. These showed the ipso carbon in their ^{13}C spectra at δ 66.3 for 16, at δ 71.7 for 17, and at δ 82.8 for 18 with the characteristic differences in shift determined by the electronegativities of the hetero atoms attached to the carbon atoms.

Biology. Acetaminophen (1), N-methylacetaminophen (5), 2,6-dimethylacetaminophen (7), and 3,5-dimethylacetaminophen (6) were administered as an aqueous solution of the sodium salts to female Sprague–Dawley rats and Swiss mice. The drugs were administered by one of three routes (intravenous, intraperitoneal, or intragastric) as a single dose. After 48 h, kidneys and liver were taken for histological assessment of specific toxicity. Toxicity was expressed in kidney as proximal tubular necrosis deep in the cortex and in liver as centrilobular vacuolation or necrosis. Both organs were graded from 0 (normal) to 4+ (severe lesions).[4] Table I shows the assessment for the various dose levels and various routes of administration.

Acetaminophen and N-methylactaminophen were administered for comparison. With acetaminophen little tissue damage occurred in rats, whereas hepatic necrosis, with a similar dose–response to that reported,[12] was observed in mice. N-Methylacetaminophen and 2,6-dimethylacetaminophen produced little damage in either rats or mice.

3,5-Dimethylacetaminophen was slightly more nephrotoxic but showed a similar hepatotoxicity to acetaminophen. All of the drugs were more toxic to liver than to kidney, with greater damage to mouse organs than to rat organs at a given dose level. The pattern of damage in liver and in kidney was similar for all of the drugs.

Discussion

3,5-Dimethylacetaminophen is of comparable toxicity to acetaminophen, whereas 2,6-dimethylacetaminophen and N-methylacetaminophen showed very little toxicity in either rats or mice. The results for the toxicity studies on acetaminophen and N-methylacetaminophen are similar to those reported.[12]

The introduction of substitutents into a drug can markedly and often unpredictably affect the pharmacological activity or toxicity. The introduction of substituents ortho to a functional group might be expected to inhibit reactions of the group; this has not always proved to be the case. Thus, with lidocaine the two o-methyl groups substantially inhibit N-hydroxylation[19] of the amide or the amine group in the principal metabolite.[19] As well, no hydroxymethyl metabolites were reported.[20] With 2,4,6-trimethylacetanilide the o-methyl groups completely block deacetylation,[21] whereas the two o-methyl groups in 2,6-dimethylphenol do not affect the rate of sulfate formation[22] and only slightly lower the rate of glucuronide formation.[23] The effect of methyl substitution on transient reactive intermediates will also be difficult to predict. In the case of 3,5- and 2,6-dimethylactaminophen it has been possible to synthesize the corresponding N-acetyl-p-benzoquinone imines 10 and 11, respectively. If the same metabolic path as acetaminophen is followed, these quinone imines would be the reactive electrophilic toxic intermediates formed from the dimethylacetaminophens.

The N-acetyldimethyl-p-benzoquinone imines 10 and 11 are more chemically stable than the quinone imine from acetaminophen itself, and both quinone imines 10 and 11 can be isolated in crystalline form. Treatment with nucleophiles shows a distinctly different pattern of products from the two quinone imines. All the products from N-acetyl-2,6-dimethyl-p-benzoquinone imine (11) are derived from ipso addition of the nucleophile to the imine function, giving a tetrahedral carbon at C_1. In the case of water, the addition is followed by loss of acetamide to give 2,6-dimethyl-p-benzoquinone. Steric interactions of the imine function with o-methyl groups must be considerably reduced by the formation of the tetrahedral carbon and is the determining factor for the stability of the products.

In the case of N-acetyl-3,5-dimethyl-p-benzoquinone imine (10) steric hindrance is not a determining factor for,

(19) S. D. Nelson, W. L. Nelson, and W. F. Trager, J. Med. Chem., 21, 721 (1978).
(20) J. B. Keenaghan and R. N. Boyes, J. Pharmacol. Exp. Ther., 180, 454 (1972).
(21) J. H. Weisburger and C. M. Goodall, Life Sci., 7, 262 (1968).
(22) H. G. Bray, B. G. Humphris, W. V. Thorpe, K. White, and P. B. Wood, Biochem. J., 52, 419 (1952).
(23) H. G. Bray, B. G. Humphris, W. V. Thorpe, K. White, and P. B. Wood, Biochem. J., 52, 416 (1952).

while ipso attack may occur, the ultimate products are the thermodynamically more stable ones resulting from subsequent reaction or rearrangement. It can be inferred that the initial reaction is ipso addition to the imine function, since, whenever possible, replacement of the imine function occurs as in the formation of the dimethyl-*p*-benzoquinone by reaction with water and 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine by reaction with aniline. Addition to the imine function of the *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**) will be favored, since this will result in an sp^3-hybridized nitrogen and a considerable gain in resonance energy in the newly formed amide functional group. The alternative 2 position available for addition will be less favorable as the intermediate will retain the imino sp^2-hybridized nitrogen which cannot be conjugated to the carbonyl group. With suitable nucleophiles such as water or aniline, elimination of acetamide occurs to form a new double bond; however, when this course is not available, as in the case of ethanethiol, the nucleophile rearranges to the 2 position to yield the adduct.

While the ipso addition products were not observed for the *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**), it seems likely that the initial reaction of nucleophiles with *N*-acetyl-*p*-benzoquinone imines is a rapid, kinetically controlled, reversible addition to yield the ipso adduct. The adduct then rearranges to form the thermodynamically more stable compound which is ultimately isolated. The formation of such an ipso addition product could be involved in stabilizing the quinone imine and in transporting it from its site of formation to its site of action in the biological system.

From a comparison of the properties,[24,25] it can be seen that the introduction of methyl groups into the 3 and 5 positions substantially increases the stability of *N*-acetyl-*p*-benzoquinone imines. If *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine is formed from 3,5-dimethylacetaminophen, the methyl groups blocking the reactive 3 position would be expected to reduce the reactivity with both glutathione and cellular macromolecules. The decreased reactivity with glutathione will, however, increase the lifetime of the quinone imine in the biological environment, allowing the toxic molecule greater opportunity to react with key enzymes or other cellular sites. Alternatively, the lower reactivity toward nucleophilic attack may mean that oxidation of cellular components, such as cell membranes, by the quinone imine may occur more extensively, leading to cell damage.

The range of effects from nontoxic to very toxic, observed for acetaminophen and the methyl-substituted derivatives, provides a related series of compounds in which to study drug-induced toxicity. Whether the toxicity of 3,5-dimethylacetaminophen is due to metabolism via a reactive intermediate is yet to be determined. However, the variation in the toxicity of the acetaminophens, together with the differences in the reactions of the possible reactive electrophilic intermediates, suggests that the detailed study of all facets of the toxicity and metabolism of these compounds will provide a better understanding of the mechanism of metabolically induced drug toxicity.

Experimental Section

Melting points were determined in a Kofler hot-stage microscope. The NMR spectra were recorded on a Perkin-Elmer R-12, a JEOL FX-100, or a Varian HA-100 spectrometer at 60 and 100 MHz, respectively, with tetramethylsilane as internal standard. Mass spectra were obtained on an AEI MS9 high-resolution mass spectrometer at 70 eV. The peak intensity is given as a percentage of the base peak. Infrared spectra were determined as KBr disks on a Perkin-Elmer 457 spectrometer, and ultraviolet spectra were determined on a Unicam SP-800 ultraviolet spectrometer. TLC was carried out on Merck silica gel GF_{254}, 0.25 mm for analytical and 1 mm for preparative plates. Light petroleum refers to the fraction of bp 40-60 °C. Microanalyses were preformed by the Australian Microanalytical Service, Melbourne.

2,6-Dimethyl-4-nitrosophenol. 2,6-Dimethylphenol (15.26 g, 0.125 mol) was dissolved in a mixture of AcOH (12.5 mL) and EtOH (12.5 mL). The mixture was cooled to 10 °C and a saturated aqueous solution of $NaNO_2$ (15 mL, 8.7 M) was added dropwise with stirring. The temperature was maintained at between 10 and 20 °C during the course of the addition. The precipitated crude product was filtered, dried, and recrystallized from toluene (charcoal) to yield 2,6-dimethyl-4-nitrosophenol (16.4 g, 86%), mp 169–170 °C (lit.[26] 170–171 °C).

3′,5′-Dimethyl-4′-hydroxyacetanilide (6). A solution of 2,6-dimethyl-4-nitrosophenol (15.8 g, 0.10 mol) in AcOH (200 mL) and Ac_2O (11.6 mL, 0.123 mol) was treated with Adams' catalyst (100 mg) and hydrogenated under medium pressure at room temperature for 2 h. The solvent was removed after filtration, and the residue was recrystallized from water (charcoal) to yield 3′,5′-dimethyl-4′-hydroxyacetanilide (**6**; 12.9 g, 69.0%) as white needles: mp 161–162 °C; IR ν_{max} 3285 (br s, NH, OH), 1645 (s, CO), 1625 (s), 1572 (m), 1488 (s), 1420 (w), 1375 (w), 1283 (w), 1200 (s), 867 (w) cm^{-1}; ^1H NMR [$(CD_3)_2SO/CDCl_3$] δ 2.03 (s, 3 H), 2.15 (s, 6 H), 7.05 (s, 2 H), 7.19 (s, 1 H), 8.84 (br s, 1 H); ^{13}C NMR [$(CD_3)_2SO/CDCl_3$] δ 16.9 (q, $ArCH_3$), 23.7 (q, $COCH_3$), 119.7 (d, C-2′), 124.4 (s, C-3′), 131.1 (s, C-1′), 149.0 (s, C-4′), 167.6 (s, $COCH_3$); mass spectrum, *m/e* 179 (50, M), 138 (13), 137 (100, M – $COCH_2$), 136 (18). Anal. ($C_{10}H_{13}NO_2$) C, H, N.

***N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10).** To a solution of 3′,5′-dimethyl-4′-hydroxyacetanilide (1.00 g, 5.6 mmol) in dry EtOAc (200 mL) was added $Pb(OAc)_4$ (2.5 g, 5.7 mmol). The mixture was stirred rapidly at room temperature and the reaction monitored by TLC ($CHCl_3$/EtOH, 9:1). A further small quantity of $Pb(OAc)_4$ (0.3 g) was added to complete the reaction. The mixture was filtered and the solvent removed under high vacuum (1 mmHg) to give a crude pale orange-yellow residue. Four recrystallizations from ether/light petroleum gave *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**) as bright yellow needles (0.64 g, 65%): mp 114–116 °C; UV λ_{max} (*n*-hexane) 215 nm (ϵ 8340), 269 sh (24 800), 275 (25 700); IR ν_{max} 1685 (s, CH_3CO), 1638 (s, CO), 1610 (m), 1585 (s, CN), 1425 (m), 1373 (s), 1359 (m), 1318 (m), 1215 (s), 1175 (s), 920 (s) cm^{-1}; ^1H NMR (CD_2Cl_2) δ 2.01 (s, 6 H), 2.22 (s, 3 H), 6.70 (s, 2 H); ^{13}C NMR (CCl_4) δ 29.3 (q, 2 × CH_3), 36.8 (q, $COCH_3$), 125.1 (d, C-2), 135.1 (s, C-3), 143.7 (s, CN), 168.5 (s, $COCH_3$), 170.6 (s, CO); mass spectrum, *m/e* 178 (13), 177 (100, M), 162 (20, M – CH_3), 135 (19, M – CH_2CO), 134 (14), 108 (31), 106 (11). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

Reaction of *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10) with Sodium Dithionite. A solution of *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**; 65 mg, 0.367 mmol) in EtOAc (10 mL) was treated with sodium dithionite (0.1 g, 0.475 mmol) in water (2 mL). The mixture was stirred rapidly at room temperature until the color had been discharged from the organic phase. The mixture was extracted with EtOAc (3 × 15 mL), the extracts were pooled and dried ($MgSO_4$), and the solvent was removed to give 3′,5′-dimethyl-4′-hydroxyacetanilide (**6**) as a white amorphous solid (60 mg, 92%): mp 158–160 °C. A single recrystallization from chloroform/light petroleum gave needles, mp 159–160 °C, indistinguishable from an authentic sample in IR, TLC, and mass spectrum.

Reaction of *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10) with Ethanethiol. EtSH (0.2 mL, 2.66 mmol) was added to a solution of *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (100 mg, 0.565 mmol) in dry benzene (10 mL). The mixture was stirred at room temperature for 20 h. Removal of the solvent gave a residue which was subjected to preparative TLC ($CHCl_3$/EtOH,

(24) I. C. Calder, M. J. Creek, P. J. Williams, C. C. Funder, C. R. Green, K. N. Ham, and J. D. Tange, *J. Med. Chem.*, **16**, 499 (1973).
(25) I. C. Calder and M. J. Creek, *Aust. J. Chem.*, **29**, 1801 (1976).
(26) K. Auwers and Th. Markovits, *Ber. Dtsch. Chem. Ges.*, 41, 2332 (1908).

9.5:0.5) to yield two bands. The low R_f band gave 3′,5′-dimethyl-4′-hydroxyacetanilide (**6**; 15 mg, 15%), mp 160–162 °C, while the high R_f band was recrystallized from chloroform/light petroleum to yield 3′,5′-dimethyl-2′-(ethylthio)-4′-hydroxyacetanilide (**12**; 54 mg, 40%), mp 129.5–131 °C, as white needles: IR ν_{max} 3439 (br m), 3320 (s), 1635 (s), 1525 (s), 1270 (w), 1240 (s), 1175 (s), 980 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, J = 7.4 Hz, 3 H), 2.20 (s, 3 H), 2.24 (s, 3 H), 2.47 (s, 3 H), 2.53 (q, J = 7.4 Hz, 2 H), 5.37 (s, 1 H), 7.80 (s, 1 H), 8.04 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.6 (q), 14.7 (q), 16.5 (q, CH_2CH_3), 24.8 (q, $COCH_3$), 29.8 (t, CH_2CH_3), 119.9 (d, C-6′), 120.2 (s, C-5′), 125.2 (s, C-3′), 128.5 (s, C-2′), 133.6 (s, C-1′), 149.0 (s, C-4′), 168.1 (s, C-2); mass spctrum, m/e 239 (59, M), 197 (27, M – CH_2CO), 196 (12), 179 (11), 178 (82, M – SC_2H_5), 169 (23), 168 (100), 137 (16). Anal. ($C_{12}H_{17}NO_2S$) C, H, N, S.

Reaction of *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10) with Water. Freshly recrystallized *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**; 67 mg, 0.378 mmol) was dissolved in acetone (6 mL) and H_2O (2 mL). The solution was refluxed for 5 h, allowed to stand for a further 12 h, and then diluted with H_2O (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL), the extracts were pooled and dried ($MgSO_4$), and the solvent was removed to yield an orange crystalline solid (48 mg), mp 62–65 °C. The solid was purified by sublimation to yield 2,6-dimethyl-*p*-benzoquinone (**13**; 21 mg, 41%): mp and mmp 71–72 °C (lit.[27] 72–73 °C); identical in IR and mass spectra with an authentic sample.

Reaction of *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10) with Aniline. Aniline (0.20 mL, 2.26 mmol) was added to a stirred solution of *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine (**10**; 400 mg, 2.26 mmol) in dry CH_2Cl_2 (25 mL) at room temperature. The solvent was removed after 72 h to give an amorphous orange solid (540 mg). A portion of this residue (100 mg) was subjected twice to preparative TLC ($CHCl_3$/EtOH, 9.5:0.5, and $CHCl_3$) to give 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine (**14**) as red needles (42.7 mg, 49%): mp 105–106 °C (lit.[18] 106 °C) after recrystallization from light petroleum; UV λ_{max} (*n*-hexane) 214 nm (ϵ 5576), 227 (5950), 273 (19 274), 284 (21 519), 448 (2844); IR ν_{max} 1650 (w), 1630 (s, CO), 1608 (w), 1589 (w), 1478 (w), 1225 (w), 700 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.97 (d, J = 1.4 Hz, 3 H), 2.09 (d, J = 1.4 Hz, 3 H), 6.80–7.49 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 16.0 (q), 16.5 (q), 120.6 (d, C-2′), 124.6 (d, C-4′), 125.3 (d, C-3), 128.9 (d, C-3′), 138.0 (d, C-5), 140.9 (s, C-2), 141.9 (s, C-6), 149.8 (s, C-1′), 157.7 (s, C-4), 188.20 (s, C-1); mass spectrum, m/e 212 (21), 211 (100, M), 210 (60), 196 (32, M – CH_3), 182 (17), 168 (20), 167 (15), 108 (18), 77 (28). The product was identical in ^{1}H NMR, ^{13}C NMR, IR, and mass spectra with an authentic sample of 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine (**14**).

3,5-Dimethyl-*N*-phenyl-*p*-benzoquinone Imine (14). Nitrosobenzene and 2,6-dimethylphenol were condensed according to the procedure[18] of Ried and Neidhardt to yield 3,5-dimethyl-*N*-phenyl-*p*-benzoquinone imine (**14**), mp 105–106 °C (lit.[18] 106 °C).

Reaction of *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone Imine (10) with Ethanol. *N*-Acetyl-3,5-dimethyl-*p*-benzoquinone imine (140 mg, 0.79 mmol) was dissolved in dry Et_2O and dry EtOH (1 mL, 0.017 mol) was added. The solution was allowed to stand for 15 days at room temperature. TLC ($CHCl_3$/MeOH, 9:1) indicated mainly starting material. Removal of the solvent gave an orange-yellow solid, which was recrystallized from ether/light petroleum to give unreacted starting material (107 mg, 76%), mp 114–116 °C.

4-Amino-3,5-dimethylphenol. Sulfanilic acid (43.3 g, 0.25 mol) was diazotized and then reacted with 3,5-dimethylphenol (30.53 g, 0.25 mol) according to the procedure of Fieser.[16] The solution was stirred for 1 h, during which time the deep red azo dye precipitated. The solution was then heated to 65–70 °C and sodium dithionite (115 g) was added in small amounts until the color was discharged. Cooling the solution to 25 °C precipitated the crude aminophenol, which was filtered and dried to yield 4-amino-3,5-dimethylphenol (30.5 g, 89%). A sample was recrystallized from benzene to give white plates, mp 183–184 °C (lit.[28] 182 °C).

2′,6′-Dimethyl-4′-hydroxyacetanilide (7). 4-Amino-3,5-dimethylphenol (9.0 g, 0.66 mol) was dissolved in H_2O (150 mL) which contained concentrated hydrochloric acid (5.5 mL). The solution was heated with stirring to 50 °C, charcoal (2 g) was added, and the mixture was stirred for 5 min. The mixture was then filtered and NaOAc (10 g) in H_2O (30 mL) was added together with Ac_2O (8 mL). The mixture was stirred rapidly and cooled in an ice bath for 1 h. 2′,6′-Dimethyl-4′-hydroxyacetanilide (**7**; 10.5 g, 89%) precipitated as off-white plates. A sample was recrystallized from $CHCl_3$/light petroleum to give needles: mp 182.5–183.5 °C; IR ν_{max} 3318 (s, NH), 3200 (br m, OH), 1638 (s, CO), 1595 (m), 1535 (s), 1469 (s), 1435 (w), 1318 (w), 1297 (s), 1253 (w), 1207 (w), 1150 (s) cm^{-1}; ^{1}H NMR [$(CD_3)_2SO$] δ 1.98 (s, 3 H), 2.02 (s, 6 H), 6.44 (s, 2 H), 8.93 (br s, 1 H), 9.12 (s, 1 H); ^{13}C NMR [$CDCl_3$/$(CD_3)_2SO$] δ 18.3 (q, $ArCH_3$), 22.6 (q, $COCH_3$), 114.4 (d, C-3′), 126.3 (s, C-2′), 136.4 (s, C-1′), 155.6 (s, C-4′), 169.3 (s, $COCH_3$); mass spectrum, m/e 180 (13), 179 (86, M), 138 (24), 137 (100, M – $COCH_2$), 136 (67), 122 (20). Anal. ($C_{10}H_{13}NO_2$) C, H, N.

2,6-Dimethyl-*p*-benzoquinone (13). 2,6-Dimethylphenol was oxidized with Fremy's salt according to the procedure of Teuber and Rau[27] to yield 2,6-dimethyl-*p*-benzoquinone (**13**), mp 70–71.5 °C (lit.[27] 72–73 °C).

***N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11).** 2′,6′-Dimethyl-4′-hydroxyacetanilide (**7**; 1.0 g, 5,58 mmol) was dissolved in EtOAc (150 mL) by heating the solution to 45 °C. $Pb(OAc)_4$ (2.5 g, 5.7 mmol) was added and the solution stirred for 5 min. A further quantity of lead tetraacetate (0.1 g) was added to complete the reaction. The mixture was cooled to 5 °C, anhydrous calcium carbonate (2.0 g) was added, and the mixture was stirred for a further 5 min. The mixture was filtered, the solvent was removed in vacuo (0.1 mmHg), and the residue was taken up in ether (50 mL). The etheral solution was treated with decolorizing charcoal, refluxed for 2 min, and filtered, and the solvent was evaporated until a faint turbidity was observed upon the addition of light petroleum. Cooling the solution to −15 °C gave an orange solid, which was treated in the above manner a further five times to yield *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (**11**; 410 mg, 41%) as yellow needles: mp 71–72 °C; UV λ_{max} (*n*-hexane) 214 nm (ϵ 1840), 262 sh (25 850), 268 (26 330); IR ν_{max} 1676 (s), 1660 (s), 1632 (s), 1604 (m), 1435 (w), 1380 (w), 1359 (w), 1305 (m), 1215 (s), 1180 (w), 915 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6 H), 2.36 (s, 3 H), 6.43 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 19.31 (q, 2 × CH_3), 25.2 (q, $COCH_3$), 132.6 (d, C-2), 145.7 (s, C-3), 151.0 (s, CN), 181.1 (s, $COCH_3$), (m) 1215 (s) 1180 (w), 915 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6 H), 2.36 (s, 3 H), 6.43 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 19.31 (q, 2 × CH_3, 25.2 (q, $COCH_3$), 132.6 (d, C-2), 145.7 (s, C-3), 151.0 (s, CN), 181.1 (s, $COCH_3$), 186.4 (s, CO); mass spectrum, m/e 178 (17), 177 (100, M), 136 (12), 135 (52, M – CH_2CO), 134 (21), 107 (28), 106 (26). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Water. A solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (**11**; 70 mg, 0.39 mmol) in acetone (6 mL) and H_2O (4 mL) was refluxed for 6 h. The solvent was removed to give a crude yellow solid (52 mg), mp 64–67 °C, which was sublimed to give 2,6-dimethyl-*p*-benzoquinone (**13**; 41 mg, 76%), as bright yellow needles: mp and mmp 71–72 °C (lit.[27] 72–73 °C); identical in IR and mass spectra with an authentic sample.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Aniline. A solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (**11**; 100 mg, 0.56 mmol) in ether (5 mL) was added dropwise over 5 min to a solution of aniline (0.52 mL, 0.56 mmol) in ether (5 mL). The solution was allowed to stand for 12 h at room temperature. The crystalline product which had formed was filtered and recrystallized from $CHCl_3$/light petroleum to give 4-acetamido-4-anilino-3,5-dimethyl-2,5-cyclohexadien-1-one (**17**; 117 mg, 77%), mp 136.5–137.5 °C (dec), as pale cream needles: UV λ_{max} (CH_3CN) 214 nm sh (ϵ 12 410), 244 (27 190), 283 (5070); IR ν_{max} 3350 (br w, NH), 3000 (br w, NH), 1672 (s, CO), 1660 (s), 1632 (s, $COCH_3$), 1599 (m), 1550 (w), 1495 (s), 1432 (w), 1375 (w), 1295 (w), 1250 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 6 H), 2.12

(27) H. Teuber and W. Rau, *Chem. Ber.*, **86**, 1036 (1953).

(28) F. M. Rowe, S. H. Bannister, R. R. Seth, and R. C. Storey, *J. Soc. Chem. Ind., London*, **49**, 469T (1930).

(s, 3 H), 6.05 (br s, 1 H), 6.14 (s, 2 H), 6.53–7.24 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 18.8 (q, 2 × CH_3), 24.6 (q, $COCH_3$), 71.7 (s, C-4), 115.2 (d, C-2′), 119.9 (d, C-4′), 128.4 (d, C-2), 129.3 (d, C-3′), 143.5 (s, C-1′), 160.9 (s, C-3), 172.2 (s, $COCH_3$), 185.7 (s, C-1); mass spectrum, m/e 271 (18), 270 (M, 77), 228 (M – CH_2CO, 17), 227 (18), 199 (10), 197 (11), 196 (39), 194 (13), 184 (10), 183 (41), 182 (71), 181 (10), 180 (12), 179 (21), 178 (15), 177 (M – $ArNH_2$, 25), 168 (23), 167 (32), 143 (14), 142 (10), 137 (71), 136 (97), 135 (16), 134 (10), 128 (10), 117 (11), 108 (23), 107 (15), 106 (22), 103 (16), 93 (100), 94 (63). Anal. ($C_{16}H_{18}N_2O_2$) C, H, N.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Hydrochloric acid. Dry hydrogen chloride gas was bubbled into a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 74 mg, 0.42 mmol) in ether (15 mL). A white precipitate formed readily and after 10 min the addition of the gas was stopped. The solvent was evaporated with a stream of dry nitrogen to give a crude white amorphous solid. Recrystallization from aqueous EtOH gave 3′-chloro-2′,6′-dimethyl-4′-hydroxyacetanilide (15; 79 mg, 88%) as white prisms: mp 216.5–217.5 °C; IR ν_{max} 3290 (br s, NH, OH), 1643 (s, CO), 1582 (w), 1538 (s), 1472 (m), 1432 (w), 1370 (w), 1288 (m), 1205 (w), 1160 (m), 1029 (w) cm^{-1}; ^{1}H NMR [$CDCl_3$/$(CD_3)_2SO$] δ 2.10 (s, 3 H), 2.11 (s, 3 H), 2.20 (s, 3 H), 6.66 (s, 1 H), 8.86 (s, 1 H), 9.24 (br s, 1 H); ^{13}C NMR [$CDCl_3$/$(CD_3)_2SO$] δ 14.9 (q, $ArCH_3$), 17.3 (q, $ArCH_3$), 21.6 (q, $COCH_3$), 113.9 (d, C-5′), 117.2 (s, C-3′), 126.1 (s, C-2′, C-6′), 133.7 (s, C-1′), 150.6 (s, C-4′), 168.5 (s, $COCH_3$); mass spectrum, m/e 215 (M + 2, 22), 213 (M, 65), 173 (59), 172 (36), 171 (M – CH_2CO, 100), 170 (46), 136 (36), 107 (15), 106 (11). Anal. ($C_{10}H_{12}ClNO_2$) C, H, N, Cl.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Sodium Dithionite. Sodium dithionite (2 g, 0.10 mol) in H_2O (10 mL) was added to a rapidly stirred solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 50 mg, 0.282 mmol) in EtOAc (10 mL) at 50 °C. After 5 min the phases were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness. The residue was recrystallized from $CHCl_3$/light petroleum to yield 2′,6′-dimethyl-4′-hydroxyacetanilide (7; 50 mg, 99%), mp 183–184 °C.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Ethanethiol. Ethanethiol (0.6 mL, 7.89 mmol) was added to a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 154 mg, 0.87 mmol) in Et_2O (20 mL). A crystalline product began to form immediately and after 12 h this was filtered. Recrystallization from CH_2Cl_2/light petroleum gave 4-acetamido-3,5-dimethyl-4-(ethylthio)-2,5-cyclohexadien-1-one (16) as colorless microprisms (163 mg, 78%): mp 116.5–117.5 °C (sealed tube, with decomposition); UV λ_{max} (CH_3CN) 220 nm (ϵ 7020), 255 (10 480); IR ν_{max} 3224 (s, NH), 1655 sh (s), 1650 (s, $COCH_3$), 1625 (s, CO), 1535 (m), 1439 (m), 1375 (m), 1369 (m), 1345 (w), 1315 (w), 1292 (m), 895 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.12 (t, J = 7.5 Hz, 3 H), 2.03 (br s, 9 H), 2.10 (q, J = 7.5 Hz, 2 H), 6.00 (br s, 1 H), 6.21 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 12.4 (q, SCH_2CH_3), 18.7 (q, 2 × CH_3), 22.5 (q, $COCH_3$), 23.9 (t, SCH_2CH_3), 66.3 (s, C-4), 127.5 (d, C-2), 157.4 (s, C-3), 169.3 (s, $COCH_3$), 185.2 (s, C-1); mass spectrum, m/e 239 (22, M), 198 (10), 197 (49, M – CH_2CO), 196 (13), 179 (45), 178 (32, M – SCH_2CH_3), 177 (34), 168 (25), 138 (13), 137 (82), 136 (100), 135 (19), 134 (13), 122 (15), 108 (27), 107 (13), 106 (17). Anal. ($C_{12}H_{17}NO_2S$) C, H, N.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Ethyl Alcohol. Dry EtOH (2 mL) was added to a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 130 mg, 0.73 mmol) in Et_2O (10 mL). The mixture was allowed to stand at room temperature for a week. Removal of the solvent gave a crude white solid (163 mg), mp 153–157 °C. Recrystallization from $CHCl_3$/light petroleum gave 4-acetamido-3,5-dimethyl-4-ethoxy-2,5-cyclohexadien-1-one (18; 147 mg, 89%) as white needles: mp 161.5–162.5 °C (sealed tube, dec); UV λ_{max} (CH_3CN) 219 nm (ϵ 8650), 242 (8400), 277 (2280); IR ν_{max} 3200 (br m), 1680 (s, $COCH_3$), 1650 sh (s), 1640 (s, CO), 1535 (w), 1381 (m), 1300 (w), 1280 (w), 1172 (w), 1067 (s), 969 (w), 892 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, J = 7.2 Hz, 3 H), 1.89 (s, 3 H), 1.90 (s, 3 H), 1.99 (s, 3 H), 3.14 (q, J = 7.2 Hz, 2 H), 6.26 (s, 2 H), 6.32 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.0 (q, OCH_2CH_3), 17.1 (q, 2 × CH_3), 22.9 (q, $COCH_3$), 57.7 (t, OCH_2CH_3), 82.8 (s, C-4), 130.2 (d, C-2), 154.0 (s, C-3), 167.6 (s, $COCH_3$), 185.0 (s, C-1); mass spectrum, m/e 223 (21, M), 195 (29), 194 (100), 182 (22), 181 (19, M – CH_2CO), 180 (11), 154 (18), 153 (42), 152 (91), 138 (12), 137 (69), 136 (86), 135 (21), 125 (25), 124 (43), 112 (53), 111 (21), 110 (34), 109 (24), 108 (66), 107 (26), 106 (22). Anal. ($C_{12}H_{17}NO_3$) C, H, N.

4′-Hydroxy-*N*-methylacetanilide (5). 4-(Methylamino)-phenol sulfate (5.0 g, 0.015 mol) was dissolved in water (65 mL) and the mixture cooled with rapid stirring to 5 °C. Ac_2O (3.4 mL) was added followed, after 1 min, by a solution of NaOAc (4.4 g), in water (15 mL). 4′-Hydroxy-*N*-methylacetanilide (5; 4.2 g, 88%) precipitated as white plates. Recrystallization from CH_3OH/$CHCl_3$ gave white prisms, mp 243–245 °C (lit.[29] 245 °C).

Acknowledgment. This work was partly supported by The Australian Research Grants Committee.

(29) R. Meldola and W. F. Hollely, *J. Chem. Soc.*, **105**, 2073 (1914).

Synthesis of Certain [6:5:6] Linear Tricyclic Nucleosides as Potential Antitumor Agents

Fung-Lung Chung, Karl H. Schram, Raymond P. Panzica, Robert A. Earl, Linda L. Wotring, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, and Departments of Medicinal Chemistry and Chemistry, University of Utah, Salt Lake City, Utah 84112.
Received November 19, 1979

A new class of tricyclic nucleosides in which the aglycon has a linear [6:5:6] geometry has been synthesized using certain pyrrolo[2,3-*d*]pyrimidine nucleosides as the starting materials. An adenosine–adenosine analogue (12) has been prepared from 6-aminotoyocamycin using two different synthetic routes. An adenosine–guanosine analogue (4) and several adenosine-6-mercaptopurine ribonucleoside-type tricyclic nucleoside derivatives have also been synthesized. Structural assignments have been based on ^{1}H NMR spectral studies, as well as an unequivocal chemical proof of structure. An interesting chemical shift for the 2′ hydrogen of certain tricyclic nucleosides was observed and is discussed. The in vitro cytotoxicity of these nucleosides against leukemia L-1210 has been determined. The in vivo evaluation of these tricyclic nucleosides against mouse leukemia will also be discussed.

The synthesis of tricyclic nucleosides is a relatively new area of nucleoside research.[1,2] Isolation of the highly fluorescent nucleoside wybutosine,[3,4] determined to be residing at the 3′ end of the anticodon of yeast $tRNA^{Phe}$,

0022-2623/80/1823-1158$01.00/0