HCl in three portions, the acidic extract was basified with NH_4OH, and the resulting mixture was extracted with ether. Ether was removed from this extract under reduced pressure and the solid residue was recrystallized from petroleum ether (bp 37.6–51.2°) to give 0.55 g (59%) of **32**, mp 58–59°. *Anal.* ($C_{16}H_{24}N_2$) C, H, N.

1-[2-(1,2,3,4-Tetrahydroisoquinolyl)]-6-(1-piperidyl)-2,4-hexadiyne (33). This was prepared by the method described for **32**, utilizing 0.078 g (0.0026 mol) of paraformaldehyde, 0.16 g (0.00019 mol) of piperidine, 0.1 g of cupric acetate, and 0.25 g (0.0013 mol) of **31**. The combined reaction mixture was stirred and heated at 70° for 10 hr. Water (25 ml) was added and the resulting mixture was extracted as described for **32** to give an oil which was subjected to dry column chromatographic treatment (neutral alumina, $CHCl_3$) to provide 0.131 g (35%) of a light tan oil.

1-[2-(1,2,3,4-Tetrahydroisoquinolyl)]-6-dimethylamino-2,4-hexadiyne (34). This was prepared by the method described for **32**, utilizing 0.48 g (0.016 mol) of paraformaldehyde, 1.33 g (0.012 mol) of 1,2,3,4-tetrahydroisoquinoline, 0.2 g of cupric acetate, 0.88 g (0.0082 mol) of **22**, and 155 ml of dioxane. The combined reaction mixture was heated at 75° and stirred for 16 hr. Water (25 ml) was added and the resulting mixture was extracted as described for **32** to give a brown oil which was subjected to dry column chromatographic separation (neutral alumina, $CHCl_3$) to give 1.08 g (52%) of a straw-colored oil.

1-Dimethylamino-6-(1-pyrrolidyl)-2,4-hexadiyne (35). This was prepared by the method described for **32**, utilizing 0.88 g (0.0124 mol) of pyrrolidine, 0.495 g (0.0165 mol) of paraformaldehyde, 0.2 g of cupric acetate, 0.88 g (0.0082 mol) of **22**, and 90 ml of dioxane. This combined reaction mixture was stirred and heated at 75° for 16 hr. The product was isolated as described for **33** to give 0.60 g (38%) of a brown oil.

Bis Quaternary Ammonium Compounds. A sealed Carius tube containing the appropriate 1,6-diamino-2,4-hexadiyne and a tenfold molar excess of methyl bromide in 2-propanol was permitted to stand at room temperature for 48 hr. The resulting white precipitate was collected on a filter and recrystallized. See Table I.

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Notes

Use of Sodium Cyanoborohydride in the Preparation of Biologically Active Nitroxides

Gerald M. Rosen†

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710.

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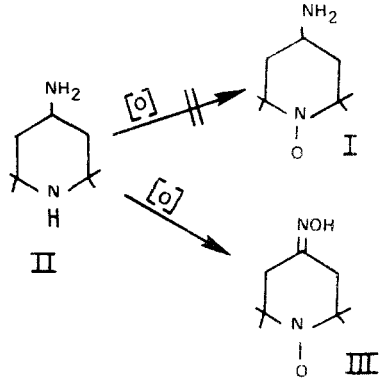
In recent years, the use of nitroxides as free-radical probes in the study of biologically significant reactions has come of age.[1-7] Unfortunately, synthetic limitations have hindered the development of many free-radical analogs of important therapeutic agents. Such is the case with 4-amino-2,2,6,6-tetramethylpiperidinooxyl (I), an intermediate in the preparation of medicinally active compounds. Rosantsev and Kokhanov[8] have found that all attempts at direct catalytic oxidation of 4-amino-2,2,6,6-tetramethylpiperidine (II) to the corresponding free radical I led to the isolation of an oxime radical III, which could not be reduced to I. The authors[8] were finally able to obtain the desired free radical I after a laborious synthetic sequence involving a reduction and oxidation. Reaction of 2,2,6,6-tetramethyl-4-piperidone (IV) with hydroxylamine gave the corresponding oxime V. Reduction using sodium in *n*-pentyl alcohol gave 4-amino-2,2,6,6-tetramethylpiperidine (VI). Subjecting the piperidine VI to acetic anhydride gave the corresponding 4-acetamido-2,2,6,6-tetramethylpiperidine (VII). Oxidation of VII with hydrogen peroxide



followed by hydrolysis gave the desired free radical I.

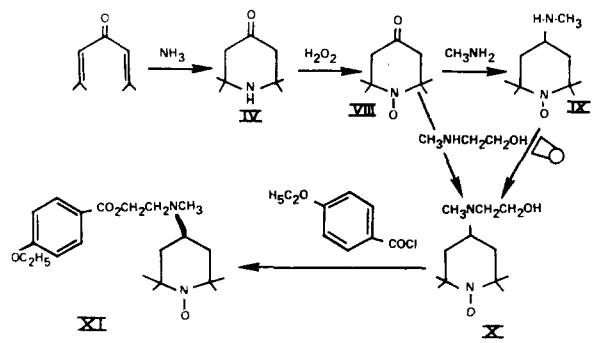
Recently, Borch, *et al.*,[9,10] have discovered the selective reductive powers of sodium cyanoborohydride. They observed that ketoximes could be reduced to the corresponding *N*-alkylhydroxylamines without overreduction to the amines. Furthermore, under mild conditions, *e.g.*, pH 6–8 and at ambient temperature, aldehydes and ketones can be reductively aminated to the corresponding primary, secondary, and tertiary amine without direct reduction of the aldehyde or ketone. When the pH is lowered to 3–4, the reduction of aldehydes or ketones is sufficiently rapid so as to make this reaction synthetically useful. With this in mind, we felt that these conditions might be mild enough to allow a reductive amination of a ketone possessing a nitroxide without eliminating the free radical. For example, the reaction of 4-oxo-2,2,6,6-tetramethylpi-

† Fellow of the Neurological Disease and Stroke Institute of the National Institutes of Health, No. 1F10 NS02697-01.

peridinooxy (VIII), ammonium acetate, and sodium cyanoborohydride at pH 7 gave 70% of the desired 4-amino-2,2,6,6-tetramethylpiperidinooxyl (I).

We found this reaction at ambient temperature to be general for most primary and secondary amines except *N*-methylaminoethanol, in which warm methanol gave the desired product. Sodium cyanoborohydride is commercially available as a brown solid. For most reductions, further purification is unnecessary. If desired, the material can be recrystallized from dioxane.[10] We have found that reductive amination of 4-oxo-2,2,6,6-tetramethylpiperidinooxy (VIII) can occur within the pH range 5–9, although the optimum yield is at pH 7–8. Furthermore, the use of 3A molecular sieves, to absorb the water, increases the yield of the product considerably.

In our continuing study of local anesthetics,[11,12] the development of a spin-labeled analog might provide us with additional information about the site of action of these agents, especially in lieu of the earlier work of Narahashi and Frazier.[13] In this communication, we discuss the synthesis and the pharmacology of 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl 4-ethoxybenzoate (XI). Warming 2,6-dimethyl-2,5-heptadien-4-one with ammonia hydroxide gave 2,2,6,6-tetramethyl-4-piperidone (IV). Oxidation of the piperidone IV with hydrogen peroxide gave the corresponding free radical VIII. Reductive amination of VIII with either methylamine followed by alkylation with ethylene oxide or warming a methanol solution of *N*-methylaminoethanol gave 4-[*N*-(2-hydroxyethyl)-*N*-methylamino]-2,2,6,6-tetramethylpiperidinooxy (X). Reaction of X with 4-ethoxybenzoyl chloride gave the local anesthetic, 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl 4-ethoxybenzoate (XI).[14]



Pharmacology. The hydrochloride salt of 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl 4-ethoxybenzoate (XI) was neutralized in physiologically buffered solution and its pharmacological action on the sciatic nerve of the frog determined. This was ascertained by placing the nerve in an isolated nerve chamber in which conducted impulses were monitored by a double-beam oscilloscope. At 1%, the compound reversibly blocked axonal conduction of the sciatic nerve.

Experimental Section

General Comments. Sodium cyanoborohydride was obtained from Alfa Products, Inc. The purification of this compound is discussed in the text. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Elemental analysis was performed by M-H-W Laboratories of Garden City, Mich. The epr spectrum was obtained on the Varian Associates E-9 spectrometer.

4-Oxo-2,2,6,6-tetramethylpiperidinooxy (VIII). To a solution containing 7.5 g of 2,2,6,6-tetramethyl-4-piperidone (IV),[15] 0.75 g of sodium tungstate, and 0.75 g of EDTA in 50 ml of H_2O was added 10 ml of 30% H_2O_2. The reaction mixture was stirred at ambient temperature for 48 hr and filtered and the pH lowered to 5. Saturation of the solution with NaCl and extraction with Et_2O gave a red oil. Chromatographic separation using neutral Al_2O_3 and CH_2Cl_2 gave 6.9 g (84%) of a red solid, mp 33–35°.[16]

4-Amino-2,2,6,6-tetramethylpiperidinooxyl (I). To a solution of 4.52 g (588 mmol) of NH_4OAc dissolved in 150 ml of absolute MeOH at pH 7–8 was added 1 g (58.8 mmol) of 4-oxo-2,2,6,6-tetramethylpiperidinooxy (VIII) and 0.258 g (41.0 mmol) of $NaBH_3CN$. The reaction was stirred at ambient temperature for 24 hr and filtered and the solvent was removed under vacuum. The remaining oil was taken up in H_2O; the pH was lowered to 5–6 with dilute HCl and extracted with $CHCl_3$. The H_2O solution was made basic with NaOH, saturated with NaCl, and extracted with $CHCl_3$. The solution was dried over anhydrous $MgSO_4$, evaporated to dryness, and chromatographed using neutral Al_2O_3 and EtOH, giving a red solid, mp 33–35°. This product was identical in all respects (ir and tlc) with the compound previously described.[8]

4-(*N*-Methylamino)-2,2,6,6-tetramethylpiperidinooxy (IX). To a solution of 2.38 g (352 mmol) of $MeNH_2$·HCl dissolved in 150 ml of absolute MeOH at pH 7–8 was added 1 g (58.8 mmol) of 4-oxo-2,2,6,6-tetramethylpiperidinooxy (VIII) and 0.222 g (35.2 mmol) of $NaBH_3CN$. The reaction was stirred at ambient temperature for 48 hr and filtered, and the solvent was removed *in vacuo*. The remaining oil was taken up in H_2O, saturated with NaCl, extracted with Et_2O, dried over anhydrous $MgSO_4$, and distilled given 0.82 g (75%), bp 56–59° (0.07 mm). *Anal.* ($C_{10}H_{21}N_2O$) C, H, N.

4-[*N*-(2-Hydroxyethyl)-*N*-methylamino]-2,2,6,6-tetramethylpiperidinooxy (X). To a solution of 1 g (5.4 mmol) of IX in 20 ml of 95% EtOH was added 3 ml of ethylene oxide. The reaction was kept at ambient temperature for 72 hr and then evaporated to dryness. The remaining oil was taken up in 50 ml of H_2O and the pH lowered to 5–6 with dilute HCl. The solution was extracted with $CHCl_3$. The H_2O layer was made basic with NaOH and extracted with $CHCl_3$. The solution was dried over anhydrous $MgSO_4$, evaporated to dryness, and chromatographed using neutral Al_2O_3 and EtOAc giving 1.7 g (73%) of a red viscous oil. The methiodide salt was prepared by dissolving some of the red oil in an Et_2O solution of MeI at ambient temperature overnight. The salt was filtered and recrystallized from absolute EtOH, mp 215–218°. *Anal.* ($C_{13}H_{28}N_2O_2I$) C, H, N.

To a solution of 2.64 g (352 mmol) of *N*-methylaminoethanol dissolved in 100 ml of absolute MeOH at pH 7–8 was added 1 g (58.8 mmol) of 4-oxo-2,2,6,6-tetramethylpiperidinooxy (VIII) and 0.222 g (35.2 mmol) of $NaBH_3CN$. The reaction was stirred at 50° for 72 hr and filtered, and the solvent was removed *in vacuo*. The work-up was identical with an earlier discussed procedure giving 0.9 g (67%) of a red viscous oil. This oil was identical in all respects (ir and tlc) with the product previously described.

2-[*N*-Methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl 4-Ethoxybenzoate (XI). To a solution of 2.48 g (10.8 mmol) of the alcohol X and 1.1 g of NEt_3 (10.8 mmol) in 40 ml of dry C_6H_6 was added 2 g (10.8 mmol) of 4-ethoxybenzoyl chloride. The reaction was stirred at ambient temperature for 12 hr and washed with 10% NaOH and then H_2O. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness leaving a red oil. This oil was chromatographed with neutral Al_2O_3 and CH_2Cl_2 giving 2.1 g (51%) of a red viscous oil, which solidified upon standing, mp 71–73°. The hydrochloride salt was prepared by dissolving a sample of the product in absolute MeOH and lowering the pH to 5–6. After the solvent had been removed, the residue was triturated with Et_2O giving the product, mp 172–174° from dioxane. *Anal.* ($C_{21}H_{34}N_2O_4Cl$) C, H, N.

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The 2′-*O*-Methyl Ether of 1-β-D-Arabinofuranosylcytosine†

John A. Montgomery* and Anne G. Laseter

Department of Organic Chemistry, Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205.
Received October 11, 1973

1-β-D-Arabinofuranosylcytosine (ara-C)[1] and its acyl derivatives[2,3] are effective anticancer agents in animals, and ara-C has found clinical utility.[4] More recently, *O*-2,2′-cyclocytidine has also shown activity and has been shown to be resistant to deamination.[5] Ara-C owes its biologic activity largely to its interference with DNA synthesis, after its conversion to the triphosphate.[6] Its substrate and inhibitor properties result from its resemblance to 2′-deoxycytidine, indicating that the hydroxyl group at $C_{2'}$ cis to the pyrimidine does not interfere with the binding of this compound to the active sites of the enzymes that normally metabolize 2′-deoxycytidine.[7] Although it would seem logical that other substituents at $C_{2'}$ cis to the cytosine moiety would also be tolerated by these enzyme active sites, only one such structure, 2′-deoxy-2′-fluoro-β-D-arabinofuranosylcytosine, has been evaluated for anticancer activity, and it was found to be active.[7]

To examine the effect of modification of ara-C at the 2′ carbon on biologic activity, we selected the 2′-*O*-methyl ester **7** for synthesis. This compound was produced in 3% yield, along with six other products, by the dimethyl sulfate–aqueous base methylation of ara-C.[8] Since this hardly seemed a practical approach for the preparation of enough material for biologic evaluation, another procedure was sought. Methylation of 3′,5′-di-*O*-dibutyryl-ara-C[3] by diazomethane in dimethoxyethane seemed promising, since ribonucleosides can be selectively alkylated at the 2′-hydroxyl in this manner.[9] In the present case, no reaction occurred in 24 hr. The addition of boron trifluoride etherate caused reaction to occur, but the product isolated in 25% yield and identified by spectral data was a mixture of two O-methylated nucleosides of uracil.

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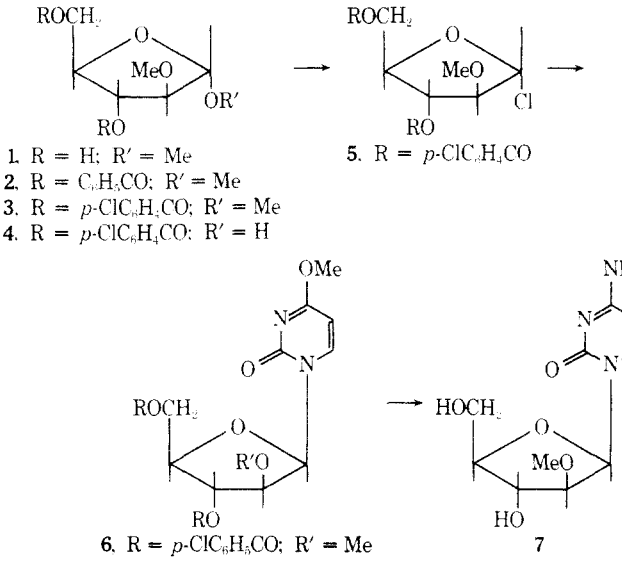
The failure of these methylation procedures led us to seek another approach. Austin, *et al.*,[10] found that, although nucleophilic attack on methyl 2,3-anhydro-β-D-ribofuranoside occurs predominantly at C-3, sodium methoxide attacks the α anomer exclusively at C-2. We have confirmed these results, obtaining a good yield of methyl 2-*O*-methyl-α-D-arabinofuranoside (**1**) by this reaction with no chromatographic or pmr spectra evidence for the formation of the 3-*O*-methyl xylo isomer.‡ Treatment of the resulting methyl 2-*O*-methyl-α-D-arabinofuranoside (**1**) with either benzoyl or *p*-chlorobenzoyl chloride in pyridine gave the 3,5-dibenzoylated sugars **2** and **3**. Hydrolysis of **3** gave 3,5-di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-D-arabinose (**4**), which was chlorinated with hydrogen chloride in methylene chloride. Heating a neat mixture of the chloro sugar **5** and 3,4-dimethoxypyrimidine gave a single nucleoside, identified by a nuclear Overhauser pmr experiment as the β or cis anomer **6** in good yield. The *p*-chlorobenzoyl groups were removed with methanolic ammonia, which also replaced the 4-methoxy group to give the desired 1-(2-*O*-methyl-β-D-arabinofuranosyl)cytosine (**7**) (Scheme I). Unfortunately, this nucleoside failed to show cytotoxicity to H.Ep.-2 cells in culture or to inhibit the L1210 leukemia *in vivo*. The reasons for this failure are not yet known.

Scheme I



1. R = H; R′ = Me
2. R = C_6H_5CO; R′ = Me
3. R = *p*-ClC_6H_4CO; R′ = Me
4. R = *p*-ClC_6H_4CO; R′ = H
5. R = *p*-ClC_6H_4CO
6. R = *p*-ClC_6H_4CO; R′ = Me
7

Experimental Section§

Methyl 2′-*O*-Methyl-α-D-arabinofuranoside (1). A solution of methyl 2,3-anhydro-α-D-ribofuranoside (146 mg, 1 mmol) and sodium methoxide (1.35 g, 25 mmol) in 5 ml of methanol was refluxed for 18 hr before it was neutralized and evaporated to dryness. The residue was extracted with acetonitrile (3 × 25 ml), and the extracts were evaporated to dryness: yield of oil, 173 mg (97%); mass spectrum 147 $(M - OCH_3)^+$; pmr ($CDCl_3$) δ 3.39 and 3.42 (2 s, 2 OMe), 3.4–4.1 (H_2, H_3, H_4, $2H_5$), 2.8–5.4 (very broad, OH), 4.9 (d, J_{12} = 1–2 Hz, H_1).

Methyl 3,5-Di-*O*-benzoyl-2-*O*-methyl-α-D-arabinofuranoside

‡Earlier work in these laboratories[11] revealed that, contrary to literature,[12,13] ammonia attacks this epoxide at C_2 and C_3 giving approximately equal amounts of the arabino and xylo isomers.

§Melting points were determined with a Mel-Temp apparatus and are not corrected. The pmr spectra were determined in the solvent indicated (Me_4Si) with a Varian XL-100-15 spectrometer, and the correct integrals were obtained for the assignments indicated; chemical shifts quoted for multiplets were measured from the approximate centers. The mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer. Chromatographic analyses were carried out on tlc plates of silica gel H (Brinkmann). The spots were detected by uv light after spraying with Ultraphor (WT, highly concentrated) and by charring after spraying with aqueous ammonium sulfate.