Synthesis and Characterization of Model Ultimate Carcinogens/Metabolites Derived from Lead Tetraacetate Oxidation of Arylnitrones: 2'-Deoxyguanosine Adducts

Honnaiah Mallesha, Kodagahally R. Ravi Kumar, Kempegowda Mantelingu, Kanchugarakoppal S. Rangappa*

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, India
Fax +91(821)518835; E-mail: rangappaks@yahoo.com

Received 9 March 2001; revised 23 April 2001

Abstract: The synthesis of model reactive metabolites **4a**–**c** by lead tetraacetate (LTA) oxidation of arylnitrones **3a**–**c** is described. Compounds **4a**–**c** react with deoxyguanosine (dG) to give *N*-benzoylated C8-adducts **5a**–**c**. Following debenzoylation with the heterogeneous system (sodium carbonate/methanol) leads to the corresponding C8-adducts **6a**–**c**.

Key words: model ultimate carcinogens, reactive metabolites, LTA oxidation, arylnitrones, debenzoylation, 2'-deoxyguanosine adducts

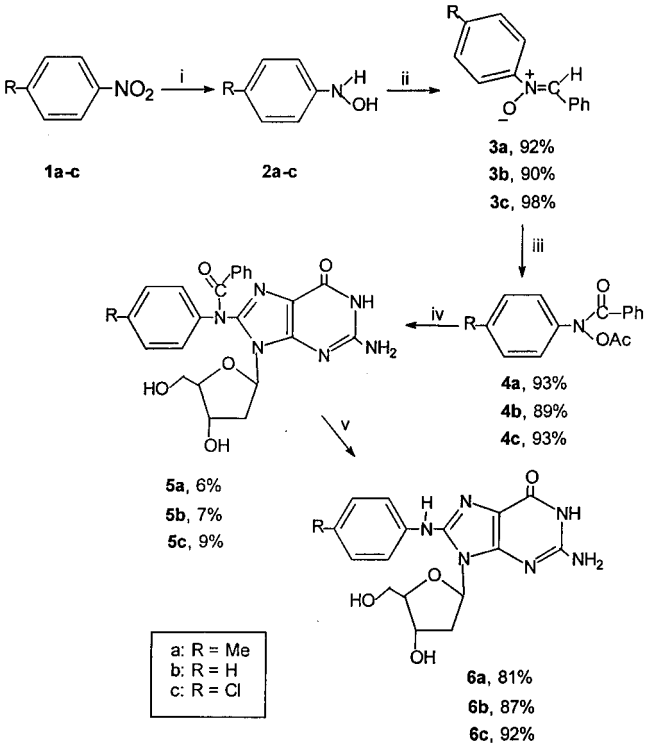
The chemistry of aromatic amines, amides and nitro compounds is of considerable interest due to their carcinogenic activity.[1] Nitrones have figured prominently in both synthetic[2–6] and biomedical science.[4,6,7] So far, most of the work on nitrone utility has concentrated on the construction of heterocyclic rings,[2] however, several examples of biologically interesting molecules have not been noted. Though nitrones and their reaction products have been identified and extensively studied in carcinogenesis by various workers,[3,8] the literature survey has indicated further scope for identification and other studies. In the interest of the biological[3,5,8] and physical properties[9] of compounds **4a**–**c**, **5a**–**c** and **6a**–**c**, it is necessary to obtain sufficient quantities by chemical synthesis. All the above, prompted us to design a new route to synthesize adducts **5a**–**c** and **6a**–**c** via arylnitrones **3a**–**c** (Scheme 1).

The *N*-hydroxyarylamines **2a**–**c**, precursors for the synthesis of **3a**–**c** were prepared by the reduction of nitro compounds **1a**–**c**. Condensation of *N*-hydroxyarylamines **2a**–**c** with benzaldehyde in ethanol gave **3a**–**c**. The nitrones **3a**–**c** were found to be light sensitive,[10] decomposing to aldehyde, amine, azobenzene, imine etc. Hence they were stored in the dark until further use.

N-Acetoxy compounds **4a**–**c** were prepared by the lead tetraacetate (LTA) oxidation[3,4,11] of **3a**–**c** in benzene. The reaction appears to proceed through the intramolecular 1,4-acetyl transfer, leads to the formation of products, **4a**–**c** (Scheme 2).

Most reactions are temperature dependent and maintained between –5 °C to 0 °C. Two of the three, **3a** and **3c** were obtained as solids after purification, while **3b** was an oil[11] after distillation under reduced pressure. Compounds **4a**–**c** were stable in a freezer for several days. IR and ^{1}H NMR spectral data have confirmed the structures of **4a**–**c**. IR absorption in the region ν = 1762–1788 cm^{-1} was assigned for ester C=O stretching frequency and the absorption at comparatively lower region ν = 1708–1714 cm^{-1} was assigned for amide C=O stretching frequency. The ^{1}H NMR peak in the region δ = 2.16–2.25 was assigned for $OCOCH_3$ protons. Other spectral data were consistent with structures of **4a**–**c**.

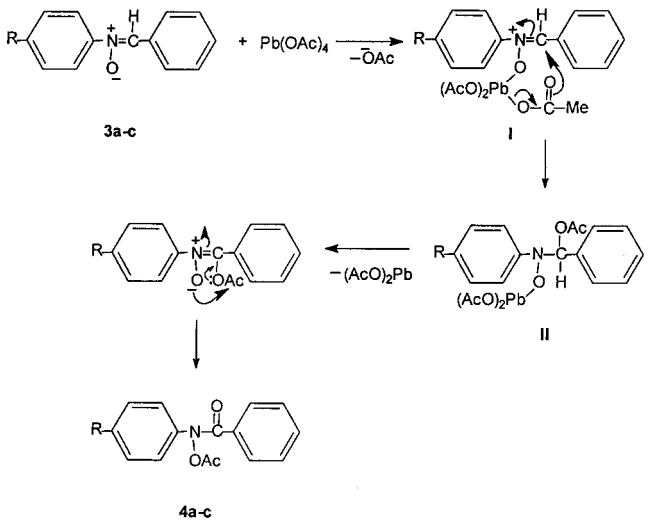
The reaction of **4a**–**c** with deoxyguanosine (dG) in sodium citrate buffer of pH 6.9 gave **5a–c**. They were confirmed by the ^{1}H NMR spectral studies. The absence of the very important peak at δ = 8.01, which was attributed to the C8-H proton in dG[12] indicated that substitution had taken



Scheme 1 *Reagents and conditions*: i) for **2a** and **2b**, Zn/NH_4Cl, distilled water, pH 6.5–7, 5% NaOH and for **2c** 5% Pd-C/THF/abs. EtOH/NH_2NH_2, 40 °C, 1 h; ii) PhCHO/EtOH or aq EtOH, r.t, 5–7 h; iii) LTA /anhyd benzene, –5 to 0 °C, then r.t., 15 min; iv) 95% EtOH, dG/2mM sodium citrate buffer of pH 6.9, 40–45 °C, 2 h, then 60–65 °C, 8 h; v) anhyd Na_2CO_3/MeOH, r.t., 7–8 h

Synthesis 2001, No. 10, 30 07 2001. Article Identifier:
1437-210X,E;2001,0,10,1459,1461,ftx,en;Z02901SS.pdf.

ISSN 0039-7881



Scheme 2

place at C8 position of the dG moiety. ^{1}H NMR spectra showed peaks in the region δ = 10.38–10.72 and 6.38–6.55 to NH and NH_2 protons, respectively, of deoxyguanosine. This indicates that there was no substituent on NH and NH_2 in the dG moiety. Absence of one NH proton in **5a**–**c** in the region δ = 8.48–8.81 assigned for **6a**–**c** confirmed the presence of a substituent on nitrogen atom of **5a**–**c**. The disappearance of all active protons in NH and OH upon the addition of D_2O indicates that, NH and OH in **5a**–**c** are free from substituent. Other spectral data were consistent with structures of **5a**–**c**.

The compounds **5a**–**c** were debenzoylated[13] to form products[14,15] **6a**–**c**. Appearance of a peak in the region δ = 8.48–8.81, attributed to the NH proton, and its disappearance upon addition of D_2O confirmed the structures of products **6a**–**c**.

In conclusion, the LTA oxidation of **3a**–**c** to give **4a**–**c**, and their reaction with dG to form C8 adducts **5a**–**c** can be summarized as follows: (i) **4a**–**c** are also reactive metabolites like other *N*-acetoxyarylamines and amides reported earlier,[14–16] (ii) nitrones **3a**–**c** are precarcinogens i.e., they essentially require activation[14] to produce the reactive metabolites **4a**–**c**. LTA is used as an excellent oxidant[3] to activate **3a**–**c** into reactive metabolites **4a**–**c**. This is a simple activation pathway to obtain **4a**–**c** via arylnitrones **3a**–**c**.

TLC was performed with 0.2 mm silica gel GF254 (E-merck) with fluorescent indication. The mobile phases used for TLC: $CHCl_3$, benzene, $CHCl_3$–hexane (7:2), Et_2O–hexane (2:1), MeOH–H_2O (7:3) and (9:1), EtOAc–hexane (2:1), MeOH, MeCN–MeOH (4:3), benzene–Et_2O (1:2). All HPLC analyses were performed with one of the mobile phases: H_2O–MeCN (6:1, 7:3 and 8.3). Compounds were obtained from the commercial sources as indicated: zin dust (E-Merck), NH_4Cl (s.d. fine), hydrazine (s.d. fine), Pd 5% on activated carbon (E-Merck), dG (Aldrich), nitrobenzene (E-Merck), 4-nitrotoluene (E-Merck), 1-chloro-4-nitrobenzene (E-Merck), LTA (Aldrich), benzaldehyde (E-Merck), silica gel-923 (Aldrich), saphadex G-15 (Aldrich), sodium citrate (Aldrich), Al (E-Merck). Melting points were recorded on SELACO 605 melting point apparatus and were uncorrected. ^{1}H NMR Spectra were recorded on Bruker AMX-400, 400Mz, NMR spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvent with TMS as an internal standard. IR spectra were recorded on a Bio-Rad Win-IR spectrometer. Elemental analyses were obtained on a Vario-EL instrument. Low temperature reactions were carried out using cryostat model MRP 700. All HPLC analysis were performed with Lachrom-2000 Merck-Hitachi L7100 pump with RP18.250-4 mm column and UV Detector-UV-VIS L7400.

Nitrones 3a–c; General Procedure

Equimolar solutions of arylhydroxylamines[17] **2a**–**c** and benzaldehyde in a minimum volume of EtOH or aq EtOH were kept at r.t. in the dark for 5–7 h. When no precipitate had formed, the solution was diluted with H_2O until milky, warmed slightly to give a clear solution and stored overnight at 0 °C. Needle like crystals obtained were recrystallized to constant mp from EtOH or aq EtOH. The analytical data of **3a**,[18] **3b**,[19] and **3c**[6] were consistent with those reported in the literature.

***N*-Acetoxy-*N*-benzoyl Derivatives 4a-c; *N*-Acetoxy-*N*-benzoylaniline (4b);[11] Typical Procedure**

A solution of **3b** (500 mg, 2.54 mmol) in anhyd benzene (10 mL) was kept between –5 °C to 0 °C. Exothermic reaction took place immediately on portion wise addition of LTA (1.60 g, 3.61 mmol). The mixture was stirred for 15 min at r.t. Filtration of the white lead diacetate precipitate and evaporation of the solvent at reduced pressure yielded the crude product. Distillation of resulting oil under reduced pressure gave **4b**.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.20 (s, 3 H, $OCOCH_3$), 7.06–7.38 (m, 5 H, NC_6H_5), 7.6–7.80 (m, 5 H, COC_6H_5).

IR (KBr): ν = 1710 (PhC=O), 1786 (CH_3C=O), 1482, 1502 (C–N), 1118 cm^{-1} (O–$COCH_3$).

Anal. Calcd for $C_{15}H_{13}NO_3$: C, 70.59; H, 5.10; N, 5.49. Found: C, 70.65; H, 5.02; N, 5.44.

***N*-Acetoxy-*N*-benzoyl-4-methylaniline (4a)**

Obtained from **3a** (500 mg, 2.37 mmol) and LTA (1.50 g, 3.38 mmol), as solid (592 mg, 93%); mp 120–122 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.16 (s, 3 H, $OCOCH_3$), 2.30 (s, 3 H, Ar-CH_3), 7.02–7.18 (m, 4 H, NC_6H_4), 7.4–7.45 (m, 5 H, COC_6H_5).

IR (KBr): ν = 1708 (PhC=O), 1762 (CH_3C=O), 1486, 1508 (C–N), 1120 cm^{-1} (O–$COCH_3$).

Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.37; H, 5.58; N, 5.20. Found: C, 71.08; H, 5.55; N, 5.28.

***N*-Acetoxy-*N*-benzoyl-4-chloroaniline (4c)**

Obtained from **3c** (500 mg, 2.61 mmol) and LTA (1.70 g, 3.83 mmol), as solid (592 mg, 93%); mp 131–132 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.25 (s, 3 H, $OCOCH_3$), 7.12–7.60 (m, 9 H, Ar-H).

IR (KBr): ν = 1714 (PhC=O), 1788 (CH_3C=O), 1488, 1502 (C–N), 1112 cm^{-1} (O–$OCOCH_3$).

Anal. Calcd for $C_{15}H_{12}NO_3$ Cl: C, 62.18; H, 4.14; N, 4.84. Found: C, 61.09; H, 4.24; N, 4.92.

dG Adducts 5a–c; *N*-(Benzoyl)-*N*-(deoxyguanosin-8-yl)-4-methylaniline (5a); Typical Procedure

Significant modifications were made to the procedure used by Kriek et al.[20] in the synthesis of Gu-adducts.

Compound **4a** (183 mg, 0.68 mmol) in 95% EtOH (15 mL) was added to dG (49 mg, 0.17 mmol) in a 2 mM sodium citrate buffer of pH 6.9 (30 mL) at 40–45 °C over 2 h and the mixture was stirred further

8 h at 60–65 °C. The reaction mixture was diluted with H_2O (50 mL) and the EtOH was evaporated. The aqueous phase was extracted successively with Et_2O (2 × 10 mL), butanol (3 × 10 mL) and EtOAc (2 × 10 mL). The combined extracts of Et_2O, butanol and EtOAc were dried (Na_2SO_4). The crude product obtained on removal of solvent was first purified over a silica gel column with MeOH–$CHCl_3$ (7:2) and then chromatographed on sephadex G-15 with EtOH–$CHCl_3$ (7:3) to give the product **5a** (6 mg, 9%). Analysis of the aqueous solution by HPLC with H_2O–MeCN (6.1) showed that the product **5a** was 99% pure.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.82 (m, 1 H, $H_2{}^1$), 2.14 (s, 3 H, Ar-CH_3), 2.60 (m, 1 H, $H_2{}^1$), 3.61 (m, 2 H, $H_5{}^1$), 3.82 (d, 1 H, $H_4{}^1$), 4.36 (m, 1 H, $H_3{}^1$), 5.28 (s, 1 H, $H_3{}^1$-OH), 5.82 (s, 1 H, $H_5{}^1$-OH), 6.24 (dd, 1 H, $H_1{}^1$), 6.38 (s, 2 H, Gu-NH_2), 6.92 (d, 2 H, Ar-H), 7.55 (d, 2 H, Ar-H), 7.59–7.82 (m, 5 H, Ar-H), 10.72 (br s, 1 H, Gu-NH).

IR (KBr): ν = 3390, 2925, 1684, 1638, 1600, 1516, 1455, 1102, 1050, 1024, 1002, 830 cm^{-1}.

Anal. Calcd for $C_{24}H_{24}N_6O_5$: C, 60.50; H, 5.04; N, 17.64. Found: C, 60.50; H, 5.04; N, 17.64.

***N*-Benzoyl-*N*-(deoxyguanosin-8-yl)aniline (5b)**

Compound **4b** (173 mg, 0.68 mmol) in 95% EtOH (15 mL) was added to dG (49 mg, 0.17 mmol) in a 2 mM sodium citrate buffer of pH 6.9 (30 mL) at 40–45 °C over 2 h and the mixture was stirred further for 9 h at 60–65 °C. The remaining procedure was the same as described for the synthesis of **5a** to obtain the product **5b** (4 mg, 6%). Analysis of the aqueous solution by HPLC with H_2O–MeCN (8:3) showed that the product **5b** was 99% pure. The spectral data were in agreement with that of an authentic sample.[14]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.02 (m, 2 H, $H_2{}^1$), 3.75 (m, 2 H, $H_5{}^1$), 3.93 (m, 1 H, $H_4{}^1$), 4.44 (m, 1 H, $H_3{}^1$), 5.35 (s, 1 H, 1 H, $H_3{}^1$-OH), 5.96 (s, 1 H, $H_5{}^1$-OH), 6.33 (m, 1 H, $H_1{}^1$), 6.45 (s, 2 H, Gu-NH_2), 6.91 (t, 1 H, Ar-H), 7.20 (t, 2 H, Ar-H), 7.72–7.82 (m, 7 H, Ar-H), 10.63 (s,1 H, Gu-NH).

IR (KBr): ν = 3345, 3206, 1686, 1600, 1564, 1498, 1355, 1098, 1052, 1030, 1000, 752 cm^{-1}.

Anal. Calcd for $C_{23}H_{22}N_6O_5$: C, 59.74; H, 4.76; N, 18.18. Found: C, 59.63; H, 4.81; N, 18.22**.**

***N*-(Benzoyl)-*N*-(deoxyguanosin-8-yl)-4-Chloroaniline (5c)**

Compound **4c** (246 mg, 0.85 mmol) in 95% EtOH (15 mL) was added to dG (49 mg, 0.17 mmol) in a 2 mM sodium citrate buffer of pH 6.9 (30 mL) at 40–45 °C over 2 h and the mixture was stirred further for 10 h at 60–65 °C. The remaining procedure was the same as described for the synthesis of **5a**, to give the product **5c** (5 mg, 7%). Analysis of the aqueous solution by HPLC with H_2O–MeCN (7:3) showed that the product **5c** was 98.9% pure.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.06 (m, 1 H, $H_2{}^1$), 2.49 (m, 1 H, $H_2{}^1$), 3.71 (m, 2 H, $H_5{}^1$), 3.92 (m, 1 H, $H_4{}^1$), 4.42 (d, 1 H, $H_3{}^1$), 5.37 (s, 1 H, $H_3{}^1$-OH), 6.04 (s, 1 H, $H_5{}^1$-OH), 6.34 (dd, 1 H, $H_1{}^1$), 6.55 (br s, 2 H, Gu-NH_2), 7.28 (d, 2 H, Ar-H), 7.58–7.74 (m, 5 H, Ar-H), 7.78 (d, 2 H, Ar-H), 10.38 (br s, 1 H, Gu-NH).

IR (KBr): ν = 3333, 2926, 1682, 1641, 1602, 1495, 1413, 1385, 1092, 1055, 1038, 1012, 826 cm^{-1}.

Anal. Calcd for $C_{23}H_{21}ClN_6O_5$: C, 55.59; H, 4.23; N, 16.92. Found: C, 55.62; H, 4.21; N, 16.99.

Debenzoylation of 5a–c to 6a–c; General Procedure

These compounds were prepared by the method of Underwood et al.[13] using heterogeneous system (Na_2CO_3/MeOH) for the debenzoylation of **5a**–**c**. Spectral data are in good agreement with an authentic sample.[14,16b]

Acknowledgement

We are grateful to the Department of Science and Technology (DST), Government of India, New Delhi, for financial support under project No. SP/S1/GO5/97.

References

(1) (a) Bartsch, H.; Dworkin, M.; Miller, J. A.; Miller, E. C. *Biochim. Biophys. Acta.* **1972**, *286*, 272. (b) Miller, E. C.; Sandin, R. B.; Miller, J. A.; Rusch, H. P. *Cancer Res.* **1956**, *16*, 525. (c) Bouck, N.; Demayorca, G. *Nature* **1976**, *264*, 722. (d) Brookers, P.; Lawley, P. D. *Nature* **1964**, *202*, 781. (e) *American Chemical Society Monograph*, No. 173; Clayson, D. B.; Garner, R. C.; Searle, C. E., Eds.; ACS: Washington, **1976**, 366. (f) Heidelberger, C. *Ann. Rev. Biochem.* **1975**, *44*, 79. (g) Kadlubar, F. F.; Miller, J. A.; Miller, E. C. *Cancer Res.* **1977**, *37*, 805. (h) Kinosita, R. *Gann.* **1936**, *30*, 423. (i) Berwald, Y.; Sachs, L. *Nature* **1963**, *200*, 1182.

(2) Padwa, A. *Intermolecular 1,3-Dipolar Cycloaddtions, In Comprehensive Organic Synthesis*, Vol. 4; Semmelhack, M. F., Ed.; Pergamon Press: Oxford, **1991**, 1069.

(3) Butler, R. N.; Scott, F. L.; O'Mahony, A. F. *Chem. Rev.* **1973**, *73*, 93.

(4) Hamer, J.; Macaluso, A. *Chem. Rev.* **1964**, *64*, 470.

(5) (a) Ohta, A.; Ochai, E. *Chem. Pharm. Bull.* **1962**, *10*, 1260. (b) Ohta, A.; Ochai, E. *Chem. Pharm. Bull.* **1963**, *11*, 1586.

(6) (a) Evans, C. A. *Aldrichim. Acta.* **1979**, *12*, 23. (b) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.

(7) (a) Barton, D. H. R.; Beaton, J. M. *J. Am. Chem. Soc.* **1960**, *82*, 2641. (b) Barton, D. H. R.; Beaton, J. M. *J. Am. Chem. Soc.* **1961**, *83*, 4083.

(8) (a) Demeunynck, M.; Lhomme, M.-F.; Mellor, J. M.; Lhomme, J. *J. Org. Chem.* **1989**, *54*, 399. (b) Demeunynck, M.; Tohme, N.; Lhomme, M.-F.; Mellor, J. M.; Lohme, J. *J. Org. Chem.* **1989**, *54*, 405.

(9) (a) Novak, M.; Rangappa, K. S. *J. Org. Chem.* **1992**, *57*, 1285. (b) Novak, M.; Rangappa, K. S.; Manitsas, R. K. *J. Org. Chem.* **1993**, *58*, 7813. (c) Novak, M.; Helmick, J. S.; Oberlies, N.; Rangappa, K. S.; Clark, W. M.; Swenton, J. S. *J. Org. Chem.* **1993**, *58*, 867.

(10) (a) Splitter, J. S.; Calvin, M. *J. Org. Chem.* **1958**, *23*, 651. (b) Kamlet, M. J.; Kaplan, L. A. *J . Org. Chem.* **1957**, *22*, 576. (c) Shindo, H.; Umezawa, B. *Chem. Pharm. Bull.* **1962**, *10*, 492.

(11) Tamagak, S.; Oae, S. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1573.

(12) Galtin, L.; Davis, J. C. *J. Am. Chem. Soc.* **1962**, *84*, 1464.

(13) Underwood, G. R.; Price, M. F.; Shapiro, R. *Carcinogenesis* **1988**, *9*, 1817.

(14) Famulok, M.; Boche, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 468.

(15) Meier, C.; Boche, G. *Chem. Ber.* **1990**, *123*, 1699.

(16) (a) Meier, C.; Boche, G. *Tetrahedron Lett.* **1990**, *31*, 1685. (b) Meier, C.; Boche, G. *Chem. Ber.* **1990**, *123*, 1991. (c) Meier, C.; Boche, G. *Tetrahedron Lett.* **1990**, *31*, 1693.

(17) (a) Patrick, T. B.; Schield, J.; Kirchner, D. G. *J. Org. Chem.* **1974**, *39*, 1758. (b) Rondestvedt, C. S.; Johnson, T. A. *Synth. Commun.* **1977**, *7*, 850.

(18) (a) Wheeler, O. H.; Gore, P. H. *J. Am. Chem. Soc.* **1956**, *78*, 3363. (b) Beckmann, E. *Liebigs Ann. Chem.* **1909**, *367*, 273.

(19) Utzinger, G. E. *Liebigs Ann. Chem.* **1944**, *556*, 50.

(20) Kriek, E.; Miller, J. A.; Juhl, U.; Miller, E. C. *Biochemistry* **1967**, *6*, 177.