then eluted with 250 ml of 1 *N* NaOH, and the eluate deposited on an 18 × 3 cm column of Dowex 50W (NH_4^+), which was washed with water until the effluent was neutral. The aqueous eluate was concentrated under reduced pressure to remove ammonia (until pH was 7–8) and then passed through a 20 × 1.4 cm column of Dowex 1-X8 (borate form), which was washed with water until all uv-absorbing material was removed. The combined eluates were concentrated to an oil which, following several evaporations from anhydrous EtOH, yielded microscopic, colorless needles of IIIa: 300 mg (85%), mp 190–192° ; uv_{max} (pH 2) 280 nm (ϵ 13,000), uv_{max} (pH 12) 271 nm (9900); chromatographically homogeneous in solvents A–E (R_f 0.26, 0.70, 0.57, 0.59, 0.28) as well as on silica gel with $CHCl_3$–MeOH (5:1). *Anal.* ($C_{10}H_{15}N_3O_5$) C, H, N.

HCl Salt of IIIa (IIIb). A solution of 40 mg (0.16 mmole) of IIIa in 2 ml of water was acidified with dilute HCl and brought to dryness, and the residue dried several times from anhydrous EtOH. Crystallization in the cold (−10°) from anhydrous EtOH-Me_2CO yielded colorless, hygroscopic prisms of IIIb: 37.5 mg (94%); mp 151–154°.

The 100-MHz nmr spectrum of IIIb, in D_2O solution, presented a unique methyl singlet at 3.45 ppm (relative to internal DSS) and, as would be expected, the protons at the 5′ position exhibited enhanced shielding, δ (H-5′, 5″) 3.77 ppm, as compared to 3.90 for the parent ara-C.

1-(5-*O*-Methyl-β-D-arabinofuranosyl)uracil (IV). A solution of 100 mg (0.39 mmole) of IIIa in 5 ml of 1 *M* AcOH was heated on a water bath. The course of deamination was followed spectrally[8] and by tlc with $CHCl_3$–MeOH (85:15, v/v). When starting product had virtually disappeared (10 hr), the solution was brought to dryness, the residue dissolved in 5 ml of water and to this was added 0.5 ml of Dowex 50W (H^+). After 15 min, the resin was filtered off and washed with water, the combined filtrates were brought to dryness, and the residue was dried from anhydrous EtOH. The compound was chromatographically homogeneous in solvent systems A–E (R_f 0.38, 0.67, 0.64, 0.50, 0.44), but its high solubility made crystallization difficult. Slow evaporation of a solution in anhydrous EtOH–Me_2CO finally yielded 15 mg of colorless, hygroscopic needles: mp 98–100°; uv_{max} (pH 2) 262 nm (ϵ 10,400), uv_{max} (pH 12) 262 (8100).

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References

(1) D. Shugar, *Virus-Cell Interact. Viral Antimetab., Fed. Eur. Biochem. Soc., Meet., 7th,* 1971, **22** (1972).
(2) D. T. Gish, R. C. Kelly, G. W. Cammiener, and W. J. Wechter, *J. Med. Chem.,* **14,** 1159 (1971).
(3) J. T. Kuśmierek and D. Shugar, *Acta Biochim. Polon.,* 18, 413 (1971).
(4) J. T. Kuśmierek, J. Giziewicz, and D. Shugar, in preparation.
(5) J. F. Codington, R. J. Cushley, and J. J. Fox, *J. Org. Chem.,* **33,** 466 (1968).
(6) T. Kanai, T. Kijima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.,* 18, 2569 (1970).
(7) J. J. Fox, N. Miller, and J. Wempen, *J. Med. Chem.,* 9, 101 (1965).
(8) R. E. Notari, M. Lue Chin, and A. Cardoni, *J. Pharm. Sci.,* **59,** 28 (1970).
(9) E. Lederer and M. Lederer, "Chromatography," Elsevier, Amsterdam, 1954.

Cotinine *N*-Oxide, a New Metabolite of Nicotine

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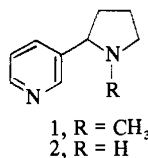
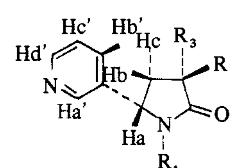
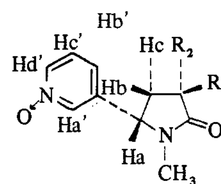
The metabolism of the tobacco alkaloid nicotine (**1**) has been studied in a variety of species,[1] including man.[2] In addition to the principle metabolites nornicotine (**2**) and cotinine (**3a**), a number of minor constituents have been reported including *N*-demethylcotinine (**4**), hydroxycotinine (**5**), and a relatively polar molecule, which in the literature has been designated compound Y.[3] During our investigations on the metabolism of cotinine in the monkey[4] we have isolated a compound with the reported chromatographic characteristics of compound Y. On the basis of spectroscopic evidence and a chemical synthesis, compound Y has been shown to be cotinine *N*-oxide (**6a**).



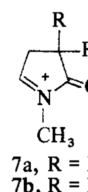
1, R = CH_3
2, R = H



3a, R_1 = CH_3; R_2 = Hd; R_3 = He
3b, R_1 = CH_3; R_2 = R_3 = D
4, R_1 = R_2 = R_2 = H
5, R_1 = CH_3; R_2 = OH; R_3 = H



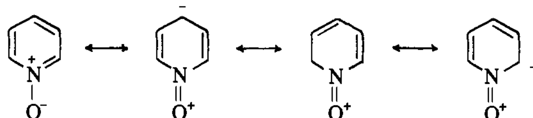
6a, R_1 = Hd; R_2 = He
6b, R_1 = R_2 = D



7a, R = H
7b, R = D

The basic fraction isolated from the urine of a 4-kg male rhesus monkey treated by intravenous infusion with *S*-cotinine showed 5 major fluorescent spots on silica gel tlc. The slowest moving spot (R_f 0.28, relative to cotinine) corresponded to literature R_f values reported for compound Y chromatographed in the same solvent system.[3] When further purification was attempted by glpc, the retention time was found to be the same as that of synthetic *S*-cotinine. Furthermore, the material collected off the glpc when submitted to high-resolution mass spectral analysis gave a parent ion with an empirical formula $C_{10}H_{12}N_2O$, isomeric with cotinine, and a mass fragmentation pattern corresponding to that of cotinine.[5] However, mass spectral analysis of compound Y purified by preparative tlc followed by alumina column chromatography gave an empirical formula $C_{10}H_{12}N_2O_2$ for the parent ion, suggesting that Y was suffering loss of oxygen when subjected to glpc. *trans*-3-Hydroxycotinine (**5**), a reported cotinine metabolite,[4] is isomeric with compound Y. However, the R_f value relative to cotinine for compound **5** is 0.60 compared to 0.28 for Y. Additionally, the mass fragmentation pattern of hydroxycotinine[4] is clearly different from metabolite Y. The spectrum of compound Y is dominated by a single fragment at *m/e* 98 which also occurs in the mass spectrum of cotinine where it has been attributed to species **7a**.[5] In independent studies on the metabolism of the dideuterated cotinine derivative **3b**, the dideuterated compound corresponding to compound Y was obtained and displayed a base peak in the mass spectra at *m/e* 100, consistent with species **7b**. The mass spectral evidence therefore suggests that the conversion of cotinine to metabolite Y involves oxidation of the pyridyl moiety. While bacteria are reported to oxidize the pyridyl group of nicotine at the C-2′ and C-6′ positions,[6] the reported oxidations by mammals of nicotine and cotinine involve alterations only of the 5-membered ring. However, mammalian systems have been reported to oxidize a number of pyridine-containing compounds to the corresponding pyridine *N*-oxides,[7] suggesting that the structure of compound Y is cotinine *N*-oxide (**6a**).

Additional evidence supporting the *N*-oxide structure was obtained from the nmr spectrum of the metabolite. The pyrrolidinone proton signals appear as multiplets centered at δ 4.5 (Ha), 2.5 (**Hb, Hd, He**), and 2.0 ppm (Hc) while the singlet for the NCH_3 group occurs at δ 2.75 ppm. The chemical shifts and multiplicities of these signals are essentially identical with those of cotinine: δ 4.6 (Ha), 2.5 (**Hb, Hd, He**), 2.1 (Hc), 2.70 ppm (NCH_3). The chemical shifts of the pyridyl proton signals of the *N*-oxide, δ 8.2 (Ha′, Hd′), 7.3 (Hc′), 7.1 ppm (Hb′) with the exception of the signal for Hc′ appear upfield relative to the corresponding cotinine signals: δ 8.6 (Ha′, Hd′), 7.3 (Hc′), and 7.6 ppm (Hb′). These upfield shifts are the expected consequence of the increased shielding of these protons due to the back polarization of the unshared electrons on oxygen as represented by the following resonance forms.[8]



The synthesis of *S*-cotinine *N*-oxide was readily accomplished by oxidation of *S*-cotinine with peroxy-*m*-chlorobenzoic acid. The ir, uv, nmr, and mass spectra of the metabolite were identical with those of the synthetic material. As was observed with the metabolite, synthetic *S*-cotinine *N*-oxide on glpc has the same retention time as *S*-cotinine. Thermal deoxygenations of *tert*-amine *N*-oxides to the corresponding *tert*-amines are well documented.[9] However, thermolysis of pyridine *N*-oxides has not been extensively investigated. Consistent with this glpc behavior we have found that heating cotinine *N*-oxide neat at 160° results in the gradual formation of cotinine together with a polymeric material.

Experimental Section†

Isolation of Metabolic Cotinine *N*-Oxide (6a). *S*-Cotinine (2.0 g), prepared by the oxidation of *S*-nicotine,[10] in 50 ml of physiological saline was administered to a 4-kg male rhesus monkey by continuous intravenous infusion over an 8-hr period. The total 48-hr urine at pH 9 was extd continuously with $CHCl_3$ for 40 hr. Silica gel tlc ($EtOH-Me_2CO-C_6H_6$–concd NH_4OH, 5:40:50:5) of the $CHCl_3$-extracted residue (1.3 g) indicated 5 major fluorescent spots with R_f values (relative to solvent front) 0.20, 0.38, 0.45, 0.51, and 0.67. The band corresponding to R_f 0.20 was eluted from prep silica gel plates (2 mm) with MeOH. Glpc of the resulting isolate on OV-17 (3 m × 30 mm, 180°) gave a material with mass spectral characteristics identical with those of *S*-cotinine. Mass spectrum: Calcd for $C_{10}H_{12}N_2O$, 176.094958. Found, 176.093806.

When the crude metabolite obtained from the silica gel plate was further purified by column chromatography on Al_2O_3 with 0.5% MeOH in $CHCl_3$, cotinine *N*-oxide was obtained as a colorless oil (20 mg): ir ($CHCl_3$), 1690 cm^{-1} (C=O), 1270 cm^{-1} (N → O);[11] uv (95% EtOH) λ_{max} 268 nm (ε 12,240), 215 (23,760);[12] Chemical ionization mass spectrum: Calcd for $C_{10}H_{13}N_2O_2$ (M + 1),‡ 193.097696. Found, 193.098079.

In a parallel experiment with *S*-3,3-dideuteriocotinine (**3b**) the *N*-oxide **6b** was isolated. Mass spectrum: Calcd for $C_{10}H_{10}D_2N_2O_2$, 194.102426. Found, 194.103270.

Synthetic *S*-Cotinine *N*-Oxide. A mixture of *S*-cotinine (1.6 g, 9.5 mmoles) and peroxy-*m*-chlorobenzoic acid[13] (1.9 g, 11 mmoles) in 50 ml of $CHCl_3$ was stirred for 18 hr at room temp. The reaction mixture was chromatographed on 50 g of Al_2O_3, eluting first with $CHCl_3$ (100 ml) and then, to obtain the product, with 2.5% MeOH in $CHCl_3$. The resulting colorless solid (2.1 g, 9.4 mmoles, 99%) was recrystd from C_6H_6 to yield pure **6a**: mp 116-117; $[\alpha]^{23}D$ −17.3 (*c* 2.52, MeOH). *Anal.* ($C_{10}H_{12}N_2O_2$) C, H, N.

†Unless otherwise specified, all reactions were performed under a N_2 atmosphere. Organic solvents were dried over anhyd $MgSO_4$ and were concd *in vacuo* by means of a rotary evaporator. Mps (Thomas-Hoover) are uncorrected. Ir spectra were taken in $CHCl_3$ on a Perkin-Elmer 337 spectrophotometer; nmr spectra were taken in $CDCl_3$ (TMS) on a Varian A-60A (δ), mass spectra were taken on an AEI MS 902 (direct inlet, 70 eV). Microanalyses were performed by the Microanalytical Labs, University of California, Berkeley, Calif.

‡This mass measurement was performed on the AEI MS-902 modified for chemical ionization using CH_4 at 1 Torr. Details of the instrumental modification will be published elsewhere.

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References

(1) H. McKennis, Jr., L. B. Turnball, and E. R. Bowman, *J. Amer. Chem. Soc.*, 79, 6342 (1957).
(2) H. McKennis, Jr., S. L. Schwartz, and E. R. Bowman, *J. Biol. Chem.*, **239**, 3990 (1969).
(3) T. Stalhandske, *Acta Physiol. Scand.*, **78**, 236 (1970).
(4) E. Dagne and N. Castagnoli, Jr., *J. Med. Chem.*, **15**, 356 (1972).
(5) A. M. Duffield, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, 87, 2926 (1965).
(6) R. L. Gherna, S. H. Richardson, and S. O. Rittenberg, *J. Biol. Chem.*, 240, 3669 (1965).
(7) M. H. Bickel, *Pharmacol. Rev.*, 21, 325 (1969).
(8) R. A. Barnes, *J. Amer. Chem. Soc.*, **81**, 1935 (1959).
(9) N. Castagnoli, Jr., J. Cymerman Craig, A. P. Melikian, and S. K. Roy, *Tetrahedron*, 26, 4319 (1970).
(10) E. R. Bowman and H. McKennis, *Biochem. Prep.*, 10, 36 (1963).
(11) E. N. Shaw, "Heterocyclic Compounds, Pyridine and Its Derivatives, Part II," E. Klingsberg, Ed., Interscience, New York, N. Y., 1961, p 120.
(12) E. Ochiai, "Aromatic Amine Oxides," Elsevier, New York, N. Y., 1967, pp 126-132.
(13) J. C. Craig and K. K. Purushothaman, *J. Org. Chem.*, **35**, 1721 (1970).

Azapurine Nucleosides. 3. Synthesis of 7-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (2-Azainosine) and Related Derivatives

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The concept of modifying the purine bases found naturally in DNA and RNA has often been utilized to obtain biologically active compounds.[1] Replacement of a C atom with an N to obtain the corresponding 8-azapurine nucleosides has been the subject of a number of current research efforts.[2-4] Montgomery and Thomas[5,†] have recently reported the synthesis of 2-azaadenosine.

We wish to report a facile synthesis of 2-azainosine [7-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**2a**)] and 2-azainosinic acid [7-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one 5′-phosphate (**2c**)] from AICA-riboside [5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (**1a**)] and AICAR [5-amino-4-carboxamido-1-(β-D-ribofuranosyl)-imidazole 5′-phosphate (**1c**)], respectively (Scheme I). Initial attempts to prepare **2a** by treatment of **1a** with sodium nitrite in aqueous acetic acid[5,†] were unsuccessful and yielded only deep red dye-like material. Attempts to fluorinate **1b** by the modified Schiemann procedure[6-8] utilizing sodium nitrite in 50% fluoboric acid afforded no fluoro derivative but a small yield of 2′,3′,5′-tri-*O*-acetyl-2-azainosine (**2b**). This result suggested that stronger acid

†The first synthesis of a "2-azapurine" nucleoside was accomplished by Stevens, *et al.*[5b]