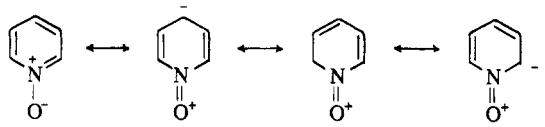
Additional evidence supporting the *N*-oxide structure was obtained from the nmr spectrum of the metabolite. The pyrrolidinone proton signals appear as multiplets centered at δ 4.5 **(Ha)**, 2.5 **(Hb, Hd, He)**, and 2.0 ppm **(Hc)** while the singlet for the NCH_3 group occurs at δ 2.75 ppm. The chemical 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.

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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).

†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.

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]

Table I. Physical Constants of 2-Azainosine Derivatives

Compd	Yield, %	Mp, °C	$[\alpha]^{25}D$	Formula[a]	Anal.[a]	Uv, nm $\lambda_{max}^{pH\ 1}$ (ϵ_{max})	Uv, nm $\lambda_{max}^{pH\ 11}$ (ϵ_{max})	pK_a
2a	86	173–175[b]	−36.5 (*c* 1, H_2O)	$C_9H_{11}N_5O_5$	C, H, N	285 (4500)	292 (6700)	6.8
2b	63	79–80	−17.6 (*c* 1, $CHCl_3$)	$C_{15}H_7N_5O_8$	C,[c] H, N	285 (4300)	292 (6500)	
2c	45	92–94	22.4 (*c* 1, H_2O)	$C_9H_{11}O_8N_5PNa\cdot 2H_2O$	C, H, N	285 (4300)	292 (6000)	
Inosine								8.9[d]

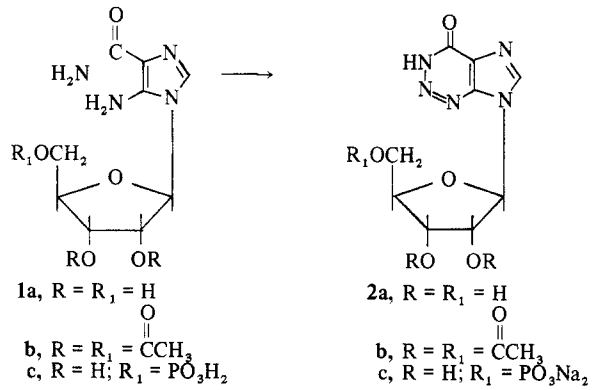
[a] Compounds were dried over P_2O_5 for 4 hr at 50° *in vacuo* before analysis. [b] Decomposed with explosion, decomn point varied slightly with rate of heating. [c] C: calcd, 45.57; found, 45.99. [d] See ref 12.

Table II. Effect of Certain 2-Azainosine Derivatives on the Multiplication of Viruses in KB Cells

Compd	Antiviral activity (VR)[a] Adenovirus	Herpes simplex	Parainfluenza virus	Rhinovirus
2a	0.0	0.0	0.6	0.0
2b	0.0	0.7	0.2	0.2
2c	0.0	0.5	0.0	0.4

[a] See ref 11.

Scheme I



conditions were necessary to suppress intermolecular diazo coupling[9] and allow ring closure. In order to suppress acidic hydrolysis of the glycosyl linkage,‡ the reaction was carried out at low temperature. The optimum conditions for ring closure were found to be treatment of the corresponding imidazole nucleosides (**1a** and **b**) in 6 *N* HCl at −25° with 3 *N* sodium nitrite to yield 2-azainosine (**2a**) and 2′,3′,5′-tri-*O*-acetyl-2-azainosine (**2b**) in yields of 86 and 63%, respectively. 2-Azainosine 5′-phosphate was conveniently prepared by the same ring closure procedure from 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (**1c**) which is readily available from 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (**1a**). The physical properties of these analogs are given in Table I. It is interesting to note that the pK_a of **2a** is more than 2 pH units lower than inosine, reflecting the effect of substitution of a N for a C atom in this fused ring system.

These compounds were tested for inhibition of herpes simplex, parainfluenza, rhino, and adenovirus[11] (Table II). 2-Azainosine (**2a**) showed activity (VR[11] = 0.6) against parainfluenza whereas 2′,3′,5′-tri-*O*-acetyl-2-azainosine (**2b**) and 2-azainosine 5′-phosphate (**2c**) inhibited the growth of herpes simplex virus in tissue culture (VRs = 0.7 and 0.5, respectively). Since these compounds showed a high degree of cytotoxicity toward KB cells (cancer cell line), cytotoxicity was determined for three cancer cell lines (KB, HEp-2, and Hela) and compared to that determined with a normal cell line (RK-13 rabbit kidney cells) (Table III). Toxicity was significantly lower against the normal cell line than against the cancer cell lines with each of these compounds.

‡ For a recent discussion of the acid-catalyzed hydrolysis of ribosyl purines see Panzica, *et al.*[10]

Table III

Compd	Cytotoxicity (highest concentration nontoxic[a] to cells, μg/ml) KB cells	HEp-2 cells	HeLa cells	RK-13 cells
2a	0.32	0.32	3.2	10.0
2b	10.0	0.32	1.00	100
2c	0.32	0.32	1.00	32.0

[a] Toxicity was determined by microscopic (200–400 X) examination; floating cells or cells with abnormal shapes or ragged edges were indicative of damaged or killed cells resulting from toxicity of the compound. Methods of tissue culture preparation are given in ref 11.

Experimental Section §

7-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (2-Azainosine, 2a). To 6 *N* HCl (50 ml) at −25° was added **1a**# (2.58 g, 10 mmoles) and the resulting soln was treated with a soln of $NaNO_2$ (2.1 g, 30 mmoles). After the reaction mixt had stirred for 30 min, the pH was carefully adjusted to 7.5 with cold, satd aqueous NaOH, while the temp of the reaction mixt was controlled below −20°. The rapidly stirred slurry was allowed to warm to room temp, and the crude product was collected by filtration, dried, and then dissolved in warm H_2O (60°, 7 ml). The pH of the soln was adjusted to 4 with concd HCl and cooled in an ice bath, and colorless needles were deposited which were collected to yield 1.37 g. The mother liquor was concd *in vacuo* to a small volume. On cooling a second crop (0.92 g) was obtained (Table I).

7-(2,3,5-Tri-*O*-Acetyl-β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (2b). To 6 *N* HCl (250 ml) at −25° was added **1b**[13] (19.2 g, 50 mmoles) and the resulting soln was treated with aqueous 3 *M* $NaNO_2$ (30 ml). After this soln was stirred for 25 min, N_2 gas was passed through the reaction mixt, and the pH was adjusted to 5 with cold, satd, aqueous NaOH, while the temp of the reaction mixt was maintained below −20°. The reaction mixt was allowed to warm to room temp and then was extracted with two portions of $CHCl_3$ (400 ml each). The $CHCl_3$ extracts were combined, dried (anhyd Na_2SO_4), and evapd to dryness *in vacuo*. The crude product was dissolved in hot EtOH (140 ml) and kept at −15° for 16 hr. The crystalline material which deposited was collected by filtration and recrystn from boiling EtOH (90 ml) (Table I).

7-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one 5′-Phosphate (2c). To 6 *N* HCl (50 ml) at −25° was added **1c**[14] (3.38 g, 10 mmoles), and the rapidly stirred soln was treated with aqueous 3 *M* $NaNO_2$ (10 ml). After the reaction mixt was stirred for 30 min,

§ Physical properties were detd with the following instruments: mp, Thomas-Hoover app (uncorrected); uv spectra, Cary 15 uv spectrometer (pH 1 and pH 11); sp rot., Perkin-Elmer Model 141 polarimeter; pmr, Hitachi Perkin-Elmer R20A high-resolution spectrometer (Me_4Si or DSS); ir spectra, Perkin-Elmer Model 257; and pK_a, Radiometer automatic titrator. Where H_2O of crystallization was indicated, this was confirmed by integration of the H_2O signal in absolute DMSO-d_6, addition of D_2O, then reintegration. Chemical analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo.

Purchased from Nutritional Biochemicals, ICN, Cleveland, Ohio.

the pH of the soln was brought to 6.2 with cold, concd, aqueous NaOH, while the temp of the reaction mixt was maintained below −20°. The temp was allowed to rise to +5° and the crystalline product was isolated by filtration and recrystn from hot H_2O. The product was washed with a small amount of cold H_2O and EtOH and then dried to yield 1.92 g (Table I).

7-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (2a) from 2b. To MeOH satd at 0° with NH_3 (150 ml) was added **2b** (5 g, 1.86 mmoles) and the mixt was stirred at room temp for 7 hr. The solvent was removed *in vacuo* to afford a yellow crystalline solid which was dried (P_2O_5 overnight). This residue was triturated with CH_3CN (50 ml), collected, washed with CH_3CN (30 ml), and then dried to yield 3.3 g. This was dissolved in H_2O (50 ml), and the soln was acidified with Dowex 50 (H^+, 100–200 mesh, 5 g) to pH 4. The resin was removed, and the filtrate was evapd to dryness to give a light yellow powder (2.6 g). This was recrystd from H_2O (18 ml) and EtOH (72 ml) to give 1.9 g of **2a**, which was identical with the product prepared by ring closure of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole.

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References

(1) L. L. Bennett and J. A. Montgomery, "Methods in Cancer Research," Vol. 3, H. Busch, Ed., Academic Press, New York, N. Y., 1967, p 549.
(2) J. A. Montgomery, H. J. Thomas, and S. J. Clayton, *J. Heterocycl. Chem.*, **7**, 215 (1970).
(3) J. A. Montgomery and H. J. Thomas, *Chem. Commun.*, 265 (1970).
(4) (a) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **36**, 1962 (1971); (b) W. Hutzenlaub, R. L. Tolman, and R. K. Robins, *J. Med. Chem.*, submitted for publication, C. W. Smith, R. W. Sidwell, R. K. Robins, and R. L. Tolman, *ibid.*, submitted for publication.
(5) (a) J. A. Montgomery and H. J. Thomas, *Chem. Commun.*, 458 (1969); (b) M. A. Stevens, H. W. Smith, and G. B. Brown, *J. Amer. Chem. Soc.*, **82**, 3189 (1960).
(6) J. F. Gerster and R. K. Robins, *ibid.*, **87**, 3752 (1965).
(7) J. A. Montgomery and K. Hewson, *ibid.*, **79**, 2259 (1957).
(8) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **33**, 432 (1968).
(9) M. R. Grimmett, *Advan. Heterocycl. Chem.*, **12**, 171 (1970).
(10) R. P. Panzica, R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, in press.
(11) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
(12) "Handbook of Biochemistry Selected Data for Molecular Biology," H. A. Sober, Ed., Chemical Rubber Co., Cleveland, Ohio, 1968, p J-98.
(13) K. Suzuki and I. Kumashiro, U. S. Patent 3,450,693 (1969); *Chem. Abstr.*, **71**, 81698 (1969).
(14) T. Saito, M. Yoshikawa, T. Meguro, K. Kusashio, T. Kato, I. Kumashiro, and T. Takenishi, Japanese Patent 6908 (1967); *Chem. Abstr.*, **68**, 40025 (1968).

Transformations in the Morphine Series. 5.[1] Reaction of Codeinone with Dimethyloxosulfonium Methylide. Structure and Analgetic Activity of the Product and Its Reduced Form

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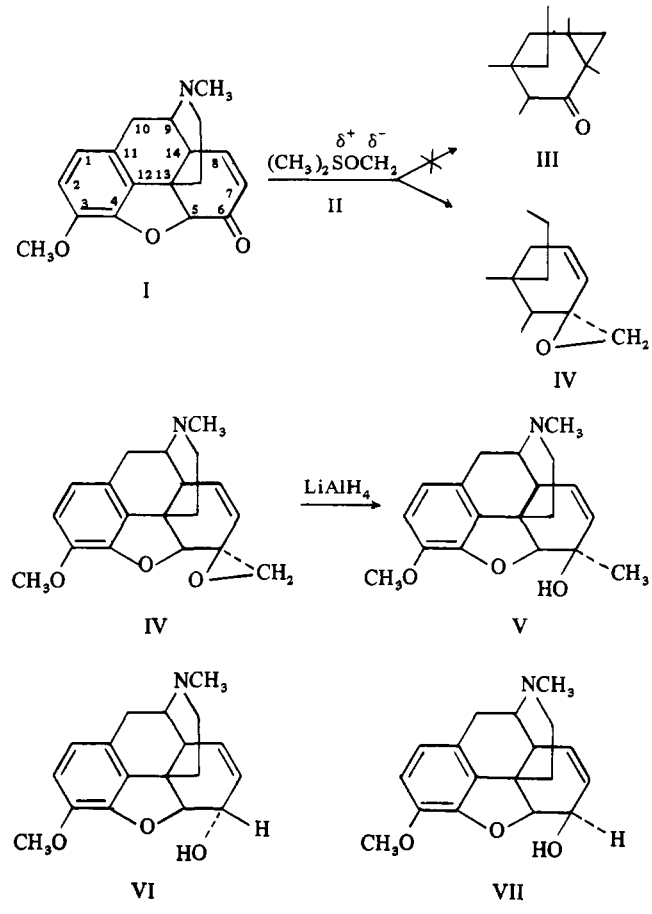
A novel oxirane derivative of codeinone was produced when the latter was interacted with dimethyloxosulfonium methylide. LAH reduction of the oxirane led to the previously unknown 6-methylisocodeine. Analgetic data for the new derivatives were determined (in mice) and compared with those of codeine, isocodeine, and 6-methylcodeine.

In exploring the preparation and utility in organic synthesis of dimethyloxosulfonium methylide, Corey and Chaykovsky[2] were able to demonstrate, in a comprehensive manner, that this ylide interacts with the carbonyl function of aromatic and nonconjugated aldehydes and ketones to form oxiranes, and with α,β-unsaturated ketones to produce cyclopropyl ketones. Both reactions were found to proceed with a high degree of stereospecificity.

In continuing our studies on transformations in the morphine series, it occurred to us that it would be of interest to study the reaction of the above oxosulfonium ylide with the α,β-unsaturated ketone system present in codeinone. Based on Corey's observations, this would be expected to yield a novel *exo*-7,8-cyclopropyl derivative with possible useful pharmacological properties.



Accordingly, when codeinone (I), prepared in good yield through silver carbonate oxidation of codeine,[3] was interacted with dimethyloxosulfonium methylide (II) (prepared *in situ* from dimethyloxosulfonium chloride[2]), the syrupy product proved to be a mixture (on silica gel tlc), consisting of a small quantity of a new substance showing the expected mass peak of 311 resulting from methylene transfer to codeinone (mass peak 297), some codeine (mass peak 299), and a quantity of noncrystallizable oil. Separation of the desired product was achieved *via* silica gel column chromatography followed by crystallization from ether. Having a homogeneous sample of the new substance in hand, we were greatly surprised to find its ir spectrum completely devoid of carbonyl absorption. Had the methylene transfer from the ylide occurred as postulated by Corey, *viz.*, addition across the double bond of the α,β-unsaturated system