After standing for 2 days at room temperature, the cyclohexane filtrate gave a white powder. Recrystallization (hexane) of this powder afforded 7.0 g (73%) of ethyl 2-carbethoxy-3-dibenzylphosphoryl-3-phenylpropionate (**22**): mp 85–86°; ir (KBr pellet) $\nu$ 1715 (C=O), 1228 (C–O), 1153 $cm^{-1}$ (P→O); pmr ($DCCl_3$) $\delta$ 0.90 (t, $CH_3$, 3 H), 1.30 (t, $CH_3$, 3 H), 2.60 (m, HCCH, 2 H), 3.24 (d, $^{31}PCH_2C_6H_5$, 2 H), 3.88 (quartet, $CH_2$, 2 H), 4.30 (m, $^{31}PCH_2C_6H_5$ and $CH_2$, 4 H), 7.20 (m, 3 $C_6H_5$, 15 H); mass spectrum (70 eV) *m/e* (rel intensity) 433 ($M^+ - C_2H_5O\cdot$, 9.0), 388 (71.0), 387 (75.6), 313 (13.2), 230 (63.8), 203 (25.3), 176 (13.2), 139 (78.2), 131 (100.0), 103 (37.9), 92 (29.5), 91 (75.2), 77 (21.1), 65 (27.7), 45 (15.6).

*Anal.* Calcd for $C_{28}H_{31}O_5P$: P, 6.47. Found: P, 6.43.

**1-Benzyl-2-phenyl-4-carbethoxy-5,5-dimethylphospholan-3-one 1-Oxide (23).** A slurry of 1.3 g (55.6% in mineral oil, 0.03 mol) of NaH in 50 ml of anhydrous THF at room temperature was treated with 6.9 g (0.03 mol) of dibenzylphosphine oxide in 50 ml of THF. The resulting mixture was heated at reflux for 15 min with vigorous evolution of a gas (presumed $H_2$) and with formation of a clear, pale-yellow solution. This solution heated to reflux was treated (dropwise) with a solution of 6.0 g (0.03 mol) of diethyl isopropylidenemalonate[19] in 75 ml of THF. After addition, the mixture was boiled for 2 hr and then treated with a slurry of 2.6 g (0.06 mol) of NaH in 50 ml of THF.

After an additional 3 hr at reflux (much gas evolved), the solution was cooled to room temperature and hydrolyzed (45 ml of 2 *N* acetic acid). The solution was concentrated to ~50 ml volume and extracted (3 × 75 ml) with $HCCl_3$. The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated to a thick yellow oil. Dissolution of this oil in boiling diethyl ether and standing for 2 days at room temperature deposited a white powder. Recrystallization of this powder from cyclohexane–chloroform (5:1) yielded 6.5 g (56.4%) of **23**: mp 171–173°; ir (KBr) $\nu$ 2540 (OH), 1717 (C=O), 1608 (–C=C–), 1107 $cm^{-1}$ (P→O); pmr ($DCCl_3$) $\delta$ 1.28 (m, $CH_3$, 9 H), 2.16 (m, CH, 1 H), 3.22 (m, $^{31}PCH_2C_6H_5$, OH, 3 H), 4.20 (m, $CH_2$, 2 H), 7.24 (m, 2 $C_6H_5$, 10 H); mass spectrum (70 eV) *m/e* (rel intensity) 384 ($M^+$, 18.0), 312 (19.9), 221 (20.9), 155 (22.3), 118 (28.1), 91 (100.0), 90 (18.0), 89 (14.4), 83 (32.4), 65 (14.4), 31 (16.2).

*Anal.* Calcd for $C_{22}H_{25}O_4P$: P, 8.06. Found: P, 8.21.

**Registry No.**—**22**, 52050-51-8; **23**, 52050-52-9; dibenzylphosphine oxide, 13238-16-9; ethyl cinnamate, 103-36-6; diethyl benzalmalonate, 5292-53-5; diethyl isopropylidenemalonate, 6802-75-1.

## References and Notes

(1) We gratefully acknowledge partial support of this work by the Public Health Service, National Cancer Institute, Grant CA 11967-10. We also acknowledge the National Science Foundation Institution grant to purchase the XL-100 nmr unit, Grant NSF GP-17641.
(2) Research Associate, 1973–1975.
(3) C. C. Chen and K. D. Berlin, *Phosphorus*, **2**, 49 (1971).
(4) T. E. Snider and K. D. Berlin, *J. Org. Chem.*, **38**, 1657 (1973), and references cited therein.
(5) (a) Some of the oxides were reported while our work was in progress; see R. Bodalski and K. Pietrusiewicz, *Tetrahedron Lett.*, 4209 (1972). (b) D. G. Smith and D. J. H. Smith, *ibid.*, 1249 (1973).
(6) These values are in very good agreement with the infrared absorption values of 2440–2275 (OH) and 1558 $cm^{-1}$ (–C=C–) for the enol form of 1-methylphospholan-3-one 1-oxide and obtained on a KBr pellet of high concentration or in Nujol mull as reported by L. D. Quin and J. A. Caputo, *Chem. Commun.*, 1463 (1968); however, the amount and absorption frequencies for OH and C=C for the enol form of 1-methylphospholan-3-one 1-oxide has recently been reported to be dependent on the medium, since as a concentrated solution in $HCCl_3$, the values were observed to be 3450 (OH) and 1590 $cm^{-1}$ (C=C) [L. D. Quin and R. C. Stock, *J. Org. Chem.*, **39**, 686 (1974)].
(7) The corresponding values of 2500 (OH) and 1587 $cm^{-1}$ (–C=C–) for the enol form of 1,3-cyclopentanedione are also in agreement: J. H. Booth, R. G. Wilkinson, S. Kushner, and J. H. Williams, *J. Amer. Chem. Soc.*, **75**, 1732 (1953).
(8) Solutions of concentrations on the order of 500 mg or less of sample in 4 ml of $DCCl_3$ did not afford the signals assigned the keto tautomer.
(9) In the present case, the existence of cis–trans isomers for a methyl function situated *β on the ring to a phosphoryl group* in phospholane 1-oxides is not considered unique. The presence of cis–trans isomers for a methyl function at C-3 of 1,3-dimethylphospholane 1-oxide and 1-phenyl-3-methylphospholane 1-oxide has been noted for but not reported from the same reaction mixture; K. L. Marsi, *Chem. Commun.*, 846 (1968); K. L. Marsi, F. B. Burns, and R. T. Clark, *J. Org. Chem.*, **37**, 238 (1972).
(10) (a) A. Bond, M. Green, and S. C. Pearson, *J. Chem. Soc. B*, 929 (1968); (b) J. G. Breen, S. I. Featherman, L. D. Quin, and R. C. Stocks, *J. Chem. Soc., Commun.*, 657 (1972).
(11) For a brief discussion on the oxidation of tertiary phosphines to phosphine oxides, see H. R. Hays and D. J. Peterson, "Organic Phosphorus Compounds," Vol. 3, G. M. Kosolapoff and L. Maier, Ed., Wiley-Interscience, New York, N. Y., 1972, Chapter 6, and references cited therein.
(12) The values of vicinal HH coupling in five-membered rings are somewhat variable but in near-planar systems $J_{cis}$ is greater than $J_{trans}$. For a review of this, see L. M. Jackman and S. Sternhell, "Applications of NMR Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Oxford, 1969, Chapter 4-2. In dihydrothiophene and dihydrofuran $J_{HCCH}$ (cis) exceeds 10 Hz and $J_{HCCH}$ (trans) is 7.5 and 8.3 Hz, respectively; see R. J. Abraham, "NMR for Organic Chemistry," D. W. Mathieson, Ed., Academic Press, New York, N. Y., 1967, Chapter 8.
(13) This value is in good agreement with that band at 1730–1740 $cm^{-1}$ observed for the carbonyl of 1-methylphospholan-3-one 1-oxide depending upon the medium (solution or a KBr pellet); see ref 6.
(14) A coupling value $J_{PCCH_3}$ = 12 Hz has also been observed for the methyl of 1,2,5-triphenyl-2-methylphosphol-3-ene 1-oxide: I. G. M. Campbell, R. C. Cookson, M. B. Hocking, and A. N. Hughes, *J. Chem. Soc.*, 2184 (1965).
(15) The complete mass spectral data for all compounds reported herein are available upon request.
(16) R. C. Miller, J. S. Bradley, and L. A. Hamilton, *J. Amer. Chem. Soc.*, **78**, 5299 (1956).
(17) R. L. Kronenthal and E. I. Becker, *J. Amer. Chem. Soc.*, **79**, 1095 (1957).
(18) C. F. H. Allen and F. W. Spangler, "Organic Syntheses," Collect. Vol. III, New York, N. Y., 1967, p 377.
(19) A. C. Cope and E. M. Hancock, *J. Amer. Chem. Soc.*, **60**, 2644 (1938).

# Purine *N*-Oxides. LVII. 9-Hydroxyhypoxanthine, Xanthine, and Guanine[1]

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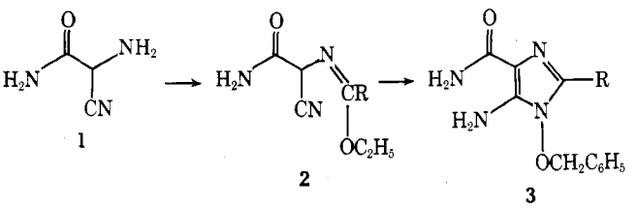
*Received April 30, 1974*

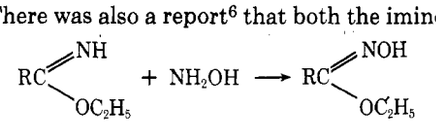
By direct application of the Shaw synthesis 8-methyl-9-hydroxypurines were successfully synthesized *via* the condensation of benzyloxyamine with the acetimidate from aminocyanoacetamide and triethyl orthoacetate. This condensation failed with triethyl orthoformate. In a modified sequence of reactions, the cyclization of the condensation product of *N*-benzyloxyformimidate and aminocyanoacetate to the imidazole was found to be catalyzed by HCl. The use of benzyloxyamine hydrochloride and the formimidate from aminocyanoacetamide also yielded the requisite imidazole. From the imidazole the title 9-hydroxypurines were obtained.

A recent synthesis of 9-hydroxy-8-methylpurines[2] involved an application of the Shaw route to 9-alkylpurines.[3] In the initial steps the condensation of triethyl orthoacetate with 2-amino-2-cyanoacetamide (**1**) to yield **2**, R = $CH_3$, was followed by condensation with benzyloxyamine to yield 5-amino-1-benzyloxy-2-methylimidazole-4-carbox-

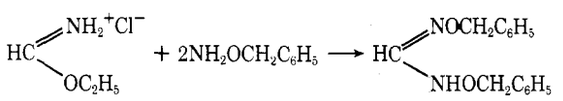


amide (3, R = $CH_3$). Attempts to extend this synthesis to 9-hydroxypurines with no substituent at position 8 failed because benzyloxyamine displaced the imino group rather than the ethoxy group from the derivative from triethyl orthoformate (2, R = H).

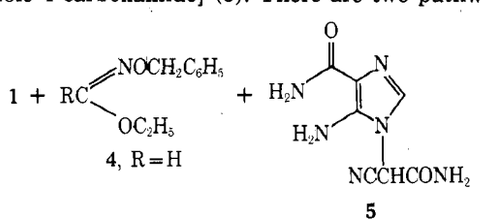
Pinner first discovered[4] that the imino group of imidiates was readily replaced by the oximino group, and Houben, *et al.*,[5] has demonstrated that good yields of hydroxamic esters could be obtained when hydroxylamine in aqueous solution was shaken with ethereal solutions of imidates. There was also a report[6] that both the imino and the



ether groups of ethyl formimidate hydrochloride were replaced by the interaction with benzyloxyamine.

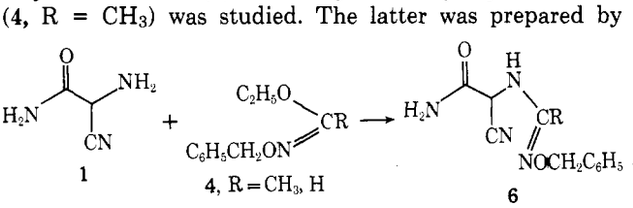


Upon further study of the reaction mixture of 2, R = H, with benzyloxyamine three products were identified, 2-amino-2-cyanoacetamide (1), ethyl *N*-benzyloxyformimidate (4, R = H), and 5-amino-1-[2-(2-cyanoacetamido)]-imidazole-4-carboxamide] (5). There are two pathways by



which the amino intermediate 1 can be regenerated, either by benzyloxyamine replacement of the imino group, giving 4, or by the direct decomposition of 2.[3] 1 then reacts with unchanged 2 to yield 5. It is evident that this reaction of benzyloxyamine is the type observed by Houben.[5]

Cook, *et al.*,[7] and Miller, *et al.*,[8] have shown that in some instances the ethoxy group of free imino ethers could react with 1 to yield imidazoles. A modification of the Shaw[3] route to 9-substituted purines was therefore explored, first to the known 5-amino-1-benzyloxy-2-methylimidazole-4-carboxamide (3, R = $CH_3$). The condensation of 2-amino-2-cyanoacetamide (1) with ethyl *N*-benzyloxyacetimidate (4, R = $CH_3$) was studied. The latter was prepared by



treating the free amine with triethyl orthoacetate. The preparation of 3, R = $CH_3$, was carried out in two steps in refluxing methanol. After several hours tlc indicated the presence of a new non-uv-absorbing spot and after 24 hr ~1 equiv of concentrated HCl was added, thus adding a minimum amount of water. The solution was again refluxed for 18 hr or until tlc showed the disappearance of the non-uv-absorbing material, presumably 6, R = $CH_3$, and the appearance of a new uv-absorbing spot which had the same $R_f$ value and uv spectrum as authentic 3, R = $CH_3$.[2] It therefore seemed possible that this procedure, with triethyl orthoformate, would yield the required imidazole with R = H. In the preparation of ethyl *N*-benzyloxyformimidate, also from the free amine and triethyl orthoformate, two products were recognized by glc of the reaction mixture. They were also separated by preparative tlc, and identified as the syn and anti isomers of 4, R = H, through their nmr spectra. It was also possible to separate these on a larger scale by column chromatography. Only the one assigned the anti configuration could be condensed with 1, under the conditions used for 4, R = $CH_3$. The imidazole 3, R = H, was obtained in a prohibitively low yield (~ 2%) by this route, but the results do show that 1 equiv of HCl facilitates the ring closure of 6 to 3, with R = $CH_3$ or H.

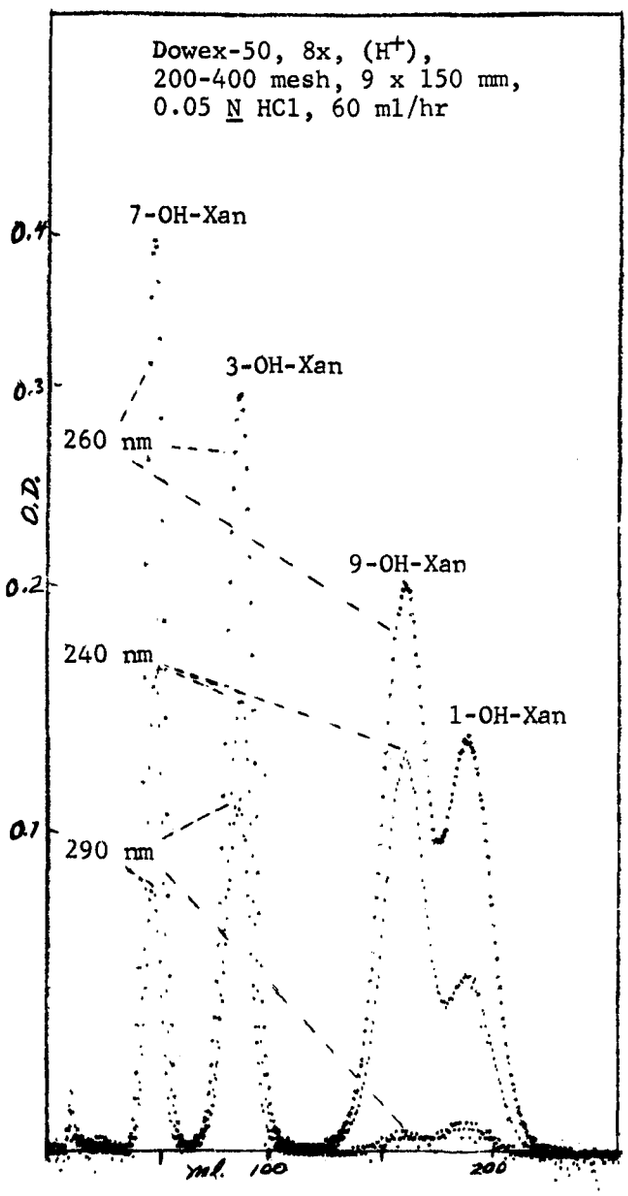
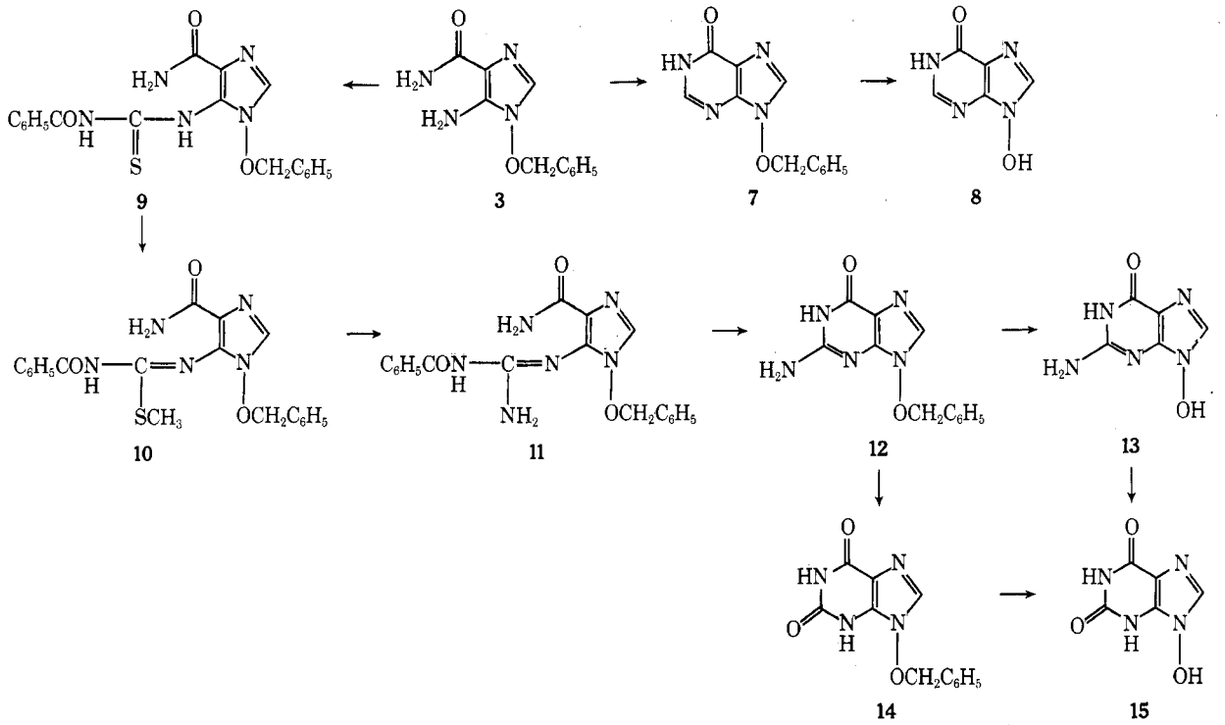



Figure 1.

The replacement of the ethoxy group of 2, R = H, with benzyloxyamine was then reinvestigated. When the hydrochloride of the latter was stirred with 2 at room temperature in ether–methanol, the proposed intermediate 6, R = H, was obtained. Upon removing the ether followed by prolonged refluxing in the methanol, the ring closure to the de-

**Scheme I**



sired 5-amino-1-benzyloxyimidazole-4-carboxamide (**3**, R = H) was brought about in an acceptable yield. No HCl other than that originally added as the benzyloxyamine hydrochloride was needed. From **3**, R = H, the desired 9-hydroxyhypoxanthine (**8**), guanine (**13**), and xanthine (**15**) were obtained by the general methods previously applied[2] to the 8-methyl derivatives. 9-Hydroxyhypoxanthine (**8**) was prepared by ring closure with ethyl formate in the presence of excess NaOEt to give 9-benzyloxyhypoxanthine (**7**), and the latter was debenzylated to 8 with HBr.

The cyclization of 5-amino-1-benzyloxyimidazole-4-carboxamide (**3**) to a 9-hydroxyguanine derivative involved refluxing with benzoyl isothiocyanate in acetone to give 5-(*N'*-benzoylthiocarbamoyl)amino-1-benzyloxyimidazole-4-carboxamide (**9**), which with methyl iodide in dilute sodium hydroxide at room temperature yielded 5-(*N'*-benzoylmethylmercaptocarbamoyl)amino-1-benzyloxyimidazole-4-carboxamide (**10**) (Scheme I). When the methyl mercaptoimidazole derivative, **10**, was heated at 100° with 2% $NH_3$ in ethanol, no displacement of the methylmercapto group by the amino group was observed, probably because of the insolubility of **10**. With $Me_2NCHO$ as the solvent[9] 5-*N'*-benzoylguanidino-1-benzyloxyimidazole-4-carboxamide (**11**) was obtained, and this was refluxed for 3 hr in 1 *N* NaOH to give a mixture of 9-benzyloxyguanine (**12**) and benzoic acid. After neutralizing with acetic acid the benzoic acid was removed by ether extraction and the 9-benzyloxyguanine was purified by recrystallization from methanol. Debenzylation yielded 9-hydroxyguanine (**13**).

Ring closure of **3**, R = H, with diethyl carbonate, as used previously for the 8-methyl derivative, did not yield 9-benzyloxyxanthine (**14**). However, by application of the nitrosation procedure[10] used to convert 1-hydroxyguanine to 1-hydroxyxanthine,[11] **12** or **13** were converted to 9-benzyloxyxanthine (**14**) and to 9-hydroxyxanthine (**15**), respectively.

This now makes available all of the possible isomeric *N*-hydroxyxanthines. A chromatogram of the four on a Dowex-50 ($H^+$) column is shown in Figure 1. Parallel assays *in vivo* are now being conducted to establish further the relationships between oncogenicity, structure, and chemical reactivities.

Certain reactivities of 9-hydroxyxanthine were compared with those known for the other isomers. Like the 3[12,13] and 7 isomers,[14] the 9 isomer yields uric acid in hot acetic anhydride. It reacts very slowly because of its insolubility, but does react readily in trifluoroacetic anhydride. The 1 isomer forms a stable 1-acetoxy derivative under these conditions.[13]

The acetoxy derivatives of the 3- and 7-hydroxyxanthines have been isolated. They react in neutral aqueous solution at room temperature to yield uric acid.[13,14] The reaction of 3-acetoxyxanthine in water has been characterized as the "3-acyloxypurine 8-substitution reaction"[15,16] which results in substitution at C-8 by many nucleophiles. In parallel, some reduction to the parent purine occurs and an insoluble blue product is also produced. The rapid reaction in water is associated with the ionization of the imidazole proton of 3-acetoxyxanthine, which can facilitate the departure of the acetoxy group from the 3 position.[16] The 7-acetoxyxanthine yields the same array of products under the same mild conditions, and a similar mechanism involving ionization of the proton at N-3 and departure of the acetoxy group from N-7 was proposed.[14] Most purine *N*-oxide derivatives are rapidly esterified by acetic anhydride in buffered aqueous solutions,[17] and the ester then reacts with any nucleophile present. Direct comparisons were made of the 3-, 7-, and 9-hydroxyxanthines under identical conditions, followed by chromatographic analyses in which uric acid, the acetoxyxanthine, 8-methylmercaptoxanthine, xanthine, and the parent *N*-hydroxyxanthine could be detected.[17] From the 3 and 7 isomers all of those products could be detected, but 9-hydroxyxanthine remained unchanged, with no evidence of any formation of an acetoxy derivative, or uric acid. Presumably a 9-acetoxy derivative is formed only under vigorous conditions, under which it yields uric acid immediately.

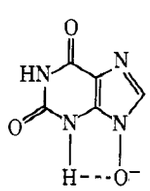
The lack of reactivity of 9-hydroxyxanthine suggests that it should not be an oncogen. Reasons for the marked contrast between the difficulty of acetylation of the 9 and the

**Table I**
**Spectral Data and p$K$ Values for 9-Hydroxypurines**

| pH | Species | $\lambda_{max}$, nm ($\epsilon \times 10^{-3}$) | | | p$K_a$ |
|---|---|---|---|---|---|
| | | 9-Hydroxyhypoxanthine | | | |
| −1 | + | | 252 (9.1) | | |
| | | | | | 0.91 ± 0.05 |
| 3.3 | 0 | 245 sh (9.1) | 250 (9.6) | | |
| | | | | | 5.18 ± 0.02[a] |
| 7.98 | − | | 235 (24.4) | | |
| | | | | | 10.61 ± 0.06 |
| 13 | −2 | | 228 (18.5)<br>277 sh (4.2) | 257 (7.5) | |
| | | 9-Hydroxyguanine | | | |
| 0 | + | 252 (10.2) | 278 (6.6) | | |
| | | | | | 2.12 ± 0.04 |
| 4.18 | 0 | 207 sh (17.0)<br>270 (7.4) | 238 (9.2) | 253 (9.5) | |
| | | | | | 5.91 ± 0.04 |
| 8.0 | −1 | 234 (24.2) | 274 (6.8) | | |
| | | | | | 10.70 ± 0.04 |
| 13.0 | −2 | 228 (14.9) | 272 (8.6) | | |
| | | 9-Hydroxyxanthine | | | |
| 2.3 | 0 | 235 (6.7) | 262 (9.8) | | |
| | | | | | 5.06 ± 0.04 |
| 6.8 | −1 | 219 (18.4) | 269 (10.9) | | |
| | | | | | 8.41 ± 0.07 |
| 11.0 | −2 | 224 (24.9) | 273 (8.1) | | |

[a] Potentiometric titration; others spectrophotometrically by methods described.[18,19]

ease of acetylation of the 7 isomer are not obvious. By analogy to previous interpretations,[20] the monoanion of 9-hydroxyxanthine shows unusually broad absorption maxima, and an incomplete expression of the strong absorption at 219–224 nm attributable to the nitrone anion (Table I). These observations are both compatible with the monoanion being a mixture of two species. Ionization of either the H of the 9-hydroxy group or of the 3-H would lead to a species which is capable of hydrogen bonding between the 3-H and the 9-N–O$^-$. If such hydrogen bonding be intermolecular, the resulting dimer could also explain the extreme insolubility in water of the 9 as compared to the 7 or 3 isomers.



## Experimental Section

Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were obtained with a Mel-Temp apparatus and are uncorrected. For thin layer chromatography (tlc) Eastman chromagram sheets with a silica gel layer containing a fluorescent indicator were used. The uv spectra were determined with a Unicam SP800A recording spectrophotometer, and nmr spectra with a Varian A-60 with $(CH_3)_2SO$-$d_6$, as solvent and $(CH_3)_4Si$ as the internal standard. Silica gel grade 923, 100–200 mesh, was used for column chromatography. The gas–liquid chromatography (glc) was carried out with an Aereograph Model A-90-P.

**Ethyl $N$-Benzyloxyacetimidate (4, R = $CH_3$).** Benzyloxyamine (6 g, 0.05 mol) and 80 ml of triethyl orthoacetate were warmed on a steam bath for ~2 hr, when tlc and glc of the reaction mixture indicated the absence of benzyloxyamine and the appearance of a new uv-absorbing material. The solvent was removed *in vacuo* and the pale yellow oil was chromatographed on silica gel with a 1:1 mixture of petroleum ether–ether. Traces of triethyl orthoacetate were eluted first, followed by ethyl $N$-benzyloxyacetimidate, which was obtained as a colorless oil upon evaporation (7.5 g, 80%): nmr $\delta$ 7.38 (s, 5, $C_6H_5CH_2$), 5.07 (s, 2, $OCH_2C_6H_5$), 4.00 (q, 2, $OCH_2CH_3$), 1.94 (s, 3, $CH_3$), 1.21 (t, 3, $CH_2CH_3$).

**5-Amino-1-benzyloxy-2-methylimidazole-4-carboxamide (3, R = $CH_3$).** 2-Amino-2-cyanoacetamide (2.5 g, 0.025 mol) and ethyl $N$-benzyloxyacetimidate (4.75 g, 0.025 mol) in methanol were refluxed with stirring for 24 hr. Tlc in 9:1 chloroform–ethanol showed a new spot when the plate was developed with iodine. An equivalent of concentrated HCl was added and the reaction mixture was refluxed for 18 hr until the tlc indicated the presence of both starting materials and the imidazole, and the absence of the non-uv-absorbing material, in that sequence. The solvent was removed *in vacuo*, leaving a dark red semisolid which was chromatographed over silica gel. Elution with chloroform gave ethyl $N$-benzyloxyacetimidate as an oil, and chloroform–ethanol (9:1) then gave **3**, R = $CH_3$, as white plates (816 mg, 13%), mp 208–209° dec (from ethanol). The uv, ir, and nmr were identical with those of an authentic specimen.[2]

**Ethyl $N$-Benzyloxyformimidate (4, R = H).** Benzyloxyamine (12 g, 0.1 mol) and 150 ml of triethyl orthoformate were warmed on a steam bath and the reaction was monitored as in **4**, R = $CH_3$. In this experiment two new uv-absorbing spots were present. The solvent was removed *in vacuo* to leave a pale yellow oil. The two major products were separated by chromatographing the oil on silica gel with petroleum ether–ether (1:1). These were identified from their nmr spectra. The first, a colorless oil (7.19 g), was assigned the anti ethyl $N$-benzyloxyformimidate structure, and the second, also a colorless oil (4.73 g), was assigned the syn structure, from their nmr: anti nmr $\delta$ 8.60 (s, 1, N=CH), 7.34 (s, 5, $CH_2C_6H_5$), 4.89 (s, 2, $OCH_2C_6H_5$), 4.00 (q, 2, $OCH_2CH_3$), 1.22 (t, 3, $CH_2CH_3$); syn nmr $\delta$ 7.34 (s, 5, $CH_2C_6H_5$), 6.83 (s, 1, N=CH), 4.90 (s, 2, $OCH_2C_6H_5$), 4.01 (q, 2, $OCH_2CH_3$), 1.20 (t, 3, $CH_2CH_3$).

**Ethyl $N$-(Carbamoylcyano)methylformimidate (2, R = H).** 2-Amino-2-cyanoacetamide (9.9 g, 0.1 mol) and 150 ml of triethyl orthoformate were stirred at 90° for ~ 3 hr, by which time tlc (chloroform–ethanol, 19:1) showed the absence of **1**, the presence of the imino ether **2**, R = H, and a uv-absorbing spot at the origin. The reaction mixture was cooled, 600 ml of petroleum ether was added, and it was kept at −10° until the precipitation was completed. The solid was collected and washed with petroleum ether. Chromatography over silica gel with chloroform–ethanol (19:1) gave the imino ether **2**, R = H, as fine crystals (6.85 g, 44%): mp 86–87° (lit.[3] mp 86–87°); nmr $\delta$ 7.99 (s, 1, N=CH), 7.61 (d, 2, $CONH_2$), 5.28 (s, 1, CH), 4.23 (q, 2, $OCH_2CH_3$), 1.25 (t, 3, $CH_2CH_3$).

**5-Amino-1-benzyloxyimidazole-4-carboxamide (3, R = H).**

**A.** Either the syn or anti **4**, R = H, isomer (4.48 g, 0.025 mol) from the foregoing and 2-amino-2-cyanoacetamide (2.5 g, 0.025 mol) in methanol were refluxed with stirring for 24 hr and the mixtures were treated as described for **3**, R = $CH_3$. The imidazole was formed only from the anti iomer, as indicated by tlc. The red oily residue obtained was chromatographed over silica gel with chloroform to yield some unreacted **4**, R = H. Elution with chloroform–ethanol (19:1) then gave 5-amino-1-benzyloxyimidazole-4-carboxamide (**3**, R = H), as white plates (113 mg, 2%), mp 168–169°.

**B.** The formimidate **2** (7.9 g, 0.05 mol) and benzyloxyamine hydrochloride (8 g, 0.05 mol) were stirred in ether–methanol (400 ml, 3:1) at room temperature for 24 hr. The white precipitate was collected and washed with 40 ml of reaction solvent to yield 4 g of 5-amino-1-[2-(2-cyanoacetamido)]imidazole-4-carboxamide, mp 212–216° (lit.[3] mp 212–216°). A tlc of the filtrate showed that it contained four products: ethyl *N*-benzyloxyformimidate (**4**), the intermediate **6**, 2-amino-2-cyanoacetamide (**1**), and traces of **5**. The ether was removed by distillation and the methanol solution was refluxed for ~18 hr or until tlc indicated complete loss of the intermediate and the formation of the required imidazole. The solvent was removed *in vacuo* to give a dark red, gum-like product which was chromatographed over silica gel with chloroform to give **4**. Elution with chloroform–ethanol (19:1) gave the imidazole. Removal of solvent and recrystallization from acetone–petroleum ether afforded white needles (1.63 g, 15%): mp 168–169°; uv $\lambda_{max}$ (EtOH) 263 nm ($\epsilon$ 11.8 × $10^3$); nmr $\delta$ 7.43 (s, 5, $CH_2C_6H_5$), 7.17 (s, 1, 2-CH), 6.71 (s, 2, $CONH_2$), 5.87 (s, 2, $CNH_2$), 5.16 (s, 2, $OCH_2C_6H_5$).

*Anal.* Calcd for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.12. Found: C, 56.71; H, 5.28; N, 24.06.

**9-Benzyloxyhypoxanthine (7).** 5-Amino-1-benzyloxyimidazole-4-carboxamide (**3**, 464 mg, 0.002 mol) and ethyl formate (1.2 ml, 0.02 mol) were refluxed for 4 hr with sodium (460 mg, 0.02 mol) in 40 ml of ethanol. Water (40 ml) was added to the cooled reaction mixture to dissolve the precipitate. When acidified with acetic acid, the 9-benzyloxyhypoxanthine precipitated and was collected and washed with water. Recrystallization from ethanol–water gave the product, **7**, as white plates (358 mg, 74%): mp 222–223°; uv $\lambda_{max}$ (pH 1) 254 nm ($\epsilon$ 12.1 × $10^3$), $\lambda_{max}$ (pH 13) 246 nm ($\epsilon$ 11.1 × $10^3$), 249 (11.8 × $10^3$); nmr $\delta$ 8.20 (s, 2, 2-CH, 8-CH), 7.50 (s, 5, $CH_2C_6H_5$), 5.46 (s, 2, $OCH_2C_6H_5$), 12.5 (br, 1, 1-NH).

*Anal.* Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.70; H, 4.30; N, 23.21.

**9-Hydoxyhypoxanthine (8).** 9-Benzyloxyhypoxanthine (**7**, 242 mg, 0.001 mol) was warmed on a steam bath in 4 ml of 32% HBr in glacial acetic acid for 3.5 hr. The reaction mixture was cooled and the HBr salt was removed by filtration and washed with several portions of ether. The product was dissolved in hot water containing a few drops of concentrated ammonia, treated with charcoal, and precipitated with acetic acid. The 9-hydroxyhypoxanthine was collected, washed with water, ethanol, and ether, and dried at 78° over $P_2O_5$ (118 mg, 79%); for uv data see Table I.

*Anal.* Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.83. Found: , 39.57; H, 2.64; N, 36.86.

**5-(*N'*-Benzoylthiocarbamoyl)amino-1-benzyloxyimidazole 4-carboxamide (9).** The imidazole **3** (1.16 g, 0.005 mol) was dissolved in hot acetone (200 ml) and 100 ml of acetone solution containing 1.1 equiv of benzoyl isothiocyanate was added. The mixture was refluxed for ~6 hr, when tlc in ethyl acetate indicated the absence of **3**. The acetone was removed *in vacuo* and the yellow oil was chromatographed on silica gel. Eluting with chloroform removed the excess benzoyl isothiocyanate. The thioureidoimidazole **9** was then eluted with chloroform–ethanol (19:1). Upon removing the solvent a yellow, gum-like product was obtained that could not be crystallized. Tlc on silica gel showed that it was chromatographically homogeneous and its nmr indicated that it was the requisite compound. This was used directly in the next step: nmr $\delta$ 8.09 (m, 2, $COC_6H_5$), 7.94 (s, 1, 2-CH), 7.66 (m, 3, $COC_6H_5$), 7.42 (s, 5, $-CH_2C_6H_5$), 5.38 (s, 2, $OCH_2C_6H_5$), 7.25 (br s, 2, $CONH_2$), 11.99 (d, 2, –NHCSNH–).

**5-(*N'*-Benzoyl-*S*-methylthiocarbamoyl)amino-1-benzyloxyimidazole-4-carboxamide (10).** The thioureidoimidazole **9** was dissolved in 0.1 *N* NaOH (100 ml) and treated with 0.5 ml of methyl iodide at room temperature. The mixture was stirred for 6 hr and the solution was then adjusted to pH 5 with glacial acetic acid and extracted several times with chloroform (100 ml). The combined chloroform extracts were dried ($Na_2SO_4$) and evaporated *in vacuo*. The residue was triturated with ethanol and collected. Recrystallization from $CHCl_3$–EtOH produced white needles of **10**: yield 623 mg (61%) based on **3**; mp 193–195°; nmr $\delta$ 7.68 (m, 5, $COC_6H_5$, 2, $CONH_2$), 7.45 (s, 1, 2-CH), 7.35 (s, 5, $CH_2C_6H_5$), 5.29 (s, 2, $OCH_2C_6H_5$), 2.51 (s, 3, $SCH_3$), 11.88 (s, 1, =CNH).

*Anal.* Calcd for $C_{20}H_{19}N_5O_3S$: C, 58.67; H, 4.68; N, 11.72; S, 7.83. Found: C, 58.61; H, 4.70; N, 11.75; S, 7.78.

**5-*N'*-Benzoylguanidino-1-benzyloxyimidazole-4-carboxamide (11).** **10** (402 mg, 0.001 mol) was treated with 50 ml of 2% $NH_3$ in $(CH_3)_2NCHO$ at 120° in a steel bomb for 6 hr, when the odor of methyl mercaptan could be recognized. The solvent was removed *in vacuo* to yield **11**. Tlc (chloroform as a solvent) showed the residue to be chromatographically pure and it was used for the preparation of **12**.

**9-Benzyloxyguanine (12).** To the glass-like residue of **11** was added 30 ml of 1 *N* NaOH and the solution was warmed on a steam bath for 3 hr. The reaction mixture was cooled and acidified to pH 5 with concentrated HCl. The white precipitate was collected and benzoic acid was removed by several extractions, or by continuous extraction, with hot ether. The solid residue was dissolved in methanol, after charcoal treatment, affording 9-benzyloxyguanine (**12**) as white plates: yield 170 mg (67%) based on **10**; uv $\lambda_{max}$ (pH 1) 255 nm ($\epsilon$ 12.4 × $10^3$), 275 (8.2 × $10^3$); $\lambda_{max}$ (pH 13) 259 sh nm ($\epsilon$ 10.8 × $10^3$), 268 (11.6 × $10^3$); nmr $\delta$ 7.61 (s, 1, 8-CH), 7.39 (s, 5, $CH_2C_6H_5$), 6.62 (br, s, 2, $NH_2$), 5.30 (s, 2, $OCH_2C_6H_5$), 11.66 (s, 1, 1-NH).

*Anal.* Calcd for $C_{12}H_{11}N_5O_2$: C, 56.02; H, 4.31; N, 27.22. Found: C, 55.85; H, 4.31; N, 27.13.

**9-Hydroxyguanine (13).** The debenzylation of **12** (129 mg, 0.0005 mol) was carried out as above. The free base was obtained from the HBr salt by dissolving in hot dilute ammonia, treatment with charcoal, and precipitation by glacial acetic acid. The white crystals of **13** were collected, washed with water, ethanol, and ether, and dried *in vacuo* over $P_2O_5$ at 78°: yield 65 mg (74%); uv data, see Table I.

*Anal.* Calcd for $C_5H_5N_5O_2 \cdot \frac{1}{2}H_2O$: C, 34.10; H, 3.43; N, 39.76. Found: C, 34.14; H, 3.32; N, 39.83.

**9-Benzyloxyxanthine (14).** A suspension of 9-benzyloxyguanine (**12**, 257 mg, 0.001 mol) in 50 ml of 2 *N* HCl was stirred at room temperature, and a solution of 2 *M* $NaNO_2$ was added dropwise for 30 min. After stirring overnight the reaction mixture was evaporated to dryness *in vacuo*, and the solid residue was dissolved in methanol–water and chromatographed on Dowex 50 ($H^+$). A trace of 9-hydroxyxanthine was eluted with 1 *N* HCl. Further elution with 2 *N* HCl gave 9-benzyloxyxanthine as a white solid. Recrystallization from acetone afforded white crystals of **14**: 155 mg (52%); uv $\lambda_{max}$ (pH 1) 238 nm ($\epsilon$ 8.1 × $10^3$), 261 (10.6 × $10^3$); $\lambda_{max}$ (pH 13) 249 nm ($\epsilon$ 10.1 × $10^3$), 276 (9.9 × $10^3$); nmr $\delta$ 7.70 (s, 1, 8-CH), 7.47 (s, 5, $CH_2C_6H_5$), 5.27 (s, 2, $OCH_2C_6H_5$), 10.81 (br s, 1, 1-NH), 12.20 (br s, 1, 3-NH).

*Anal.* Calcd for $C_{12}H_{10}N_4O_3$: C, 55.81; H, 3.90; N, 21.69. Found: C, 55.62; H, 4.13; N, 21.79.

**9-Hydroxyxanthine (15).** The debenzylation of 14 (129 mg, 0.0005 mol) was carried out. The free base, **15**, was obtained from the HBr salt by dissolving in hot dilute ammonia, treatment with charcoal, and precipitation by glacial acetic acid. The white crystals were collected, washed with water, ethanol, and ether, and dried *in vacuo* over $P_2O_5$ at 78°: yield 66 mg (73%); uv data, see Table I.

*Anal.* Calcd for $C_5H_4N_4O_3$: C, 35.72; H, 2.40; N, 33.32. Found: C, 35.54; H, 2.52; N, 33.38.

**Reaction of 9-Hydroxyxanthine with Acid Anhydrides.** **15** (10 mg) was refluxed in 5 ml of trifluoroacetic anhydride for 8 hr. After evaporation and dissolving in a few drops of NaOH, the reaction mixture was then chromatographed on Dowex 50 ($H^+$). Elution with water gave uric acid as the major product. A few micrograms of **15** in a few drops of acetic anhydride was heated to near dryness and the presence of some uric acid was shown on an analytical chromatogram.

The reactivities of the 3, 7, and 9 isomers were compared in an esterification in buffered aqueous solutions, which contained methionine, followed by analysis of the products on a standardized Dowex 50 column eluted with 0.05 *N* HCl.[17] At short reaction times, 2–3 min, the 3 and 7 isomers showed evidence of the formation of acetoxy derivatives, and after 1–16 hr showed maximal formation of 8-methylmercaptoxanthine.[21] Under both conditions 9-hydroxyxanthine remained unchanged.

**Acknowledgment.** We thank Dr. George Bosworth Brown and Dr. James C. Parham for helpful discussions, Mr. Marvin J. Olsen for assistance with the nmr spectra, and Ms. Mary Ann Caolo for the p*K* determinations.

**Registry No.**—**1**, 6719-21-7; **2** (R = H), 37842-62-9; **3** (R = $CH_3$), 34407-36-8; **3** (R = H), 51932-94-6; **4** (R = $CH_3$), 16115-53-0; *anti*-**4** (R = H), 52019-90-6; *syn*-**4** (R = H), 52019-91-7; **7**, 51932-95-7; **8**, 51932-96-8; **9**, 51932-97-9; **10**, 51932-98-0; **11**, 51932-99-1; **12**, 51933-00-7; **13**, 51933-01-8; **14**, 51933-02-9; **15**, 51933-03-0; benzyloxyamine, 622-33-3; triethyl orthoacetate, 78-39-7; triethyl orthoformate, 122-51-0; benzyloxyamine hydrochloride, 2687-43-6; ethyl formate, 109-94-4; benzoyl isothiocyanate, 532-55-8.

## References and Notes


(1) This investigation was supported in part by funds from the National Cancer Institute (Grants CA 08748 and CA 15274). Part LVI: F. L. Lam, G. B. Brown, and J. C. Parkham, *J. Org. Chem.*, in press.
(2) A. A. Watson and G. B. Brown, *J. Org. Chem.*, **37**, 1867 (1972).
(3) G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1684 (1959).
(4) A. Pinner, *Ber.*, **17**, 184, 1589 (1884).
(5) J. Houben and E. Pfankuch, *Ber.*, **59**, 2392, 2397 (1926).
(6) G. Schroeter and M. Peschkes, *Ber.*, **33**, 1975 (1900).
(7) A. H. Cook, A. C. Davis, I. Heilbron, and C. H. Thomas, *J. Chem. Soc.*, 1071 (1949).
(8) C. S. Miller, S. Gurin, and D. W. Wilson, *Science*, **112**, 654 (1950); *J. Amer. Chem. Soc.*, **74**, 1892 (1952).
(9) A. Yamazaki, I. Kumashiro, and I. Takenishi, *J. Org. Chem.*, **32**, 1826 (1967).
(10) A. A. Watson, S. C. Nesnow, and G. B. Brown, *J. Org. Chem.*, **38**, 3046 (1973).
(11) J. C. Parham, J. Fissekis, and G. B. Brown, *J. Org. Chem.*, **32**, 1151 (1967).
(12) U. Wölcke, W. Pfleiderer, T. J. Delia, aand G. B. Brown, *J. Org. Chem.*, **34**, 981 (1969).
(13) N. J. M. Birdsall, T.-C. Lee, and U. Wölcke, *Tetrahedron*, **27**, 5961 (1971).
(14) G. Zvilichovsky and G. B. Brown, *J. Org. Chem.*, **37**, 1870 (1972).
(15) N. J. M. Birdsall, U. Wölcke, T.-C. Lee, and G. B. Brown, *Tetrahedron*, **27**, 5969 (1971).
(16) N. J. M. Birdsall, J. C. Parham, U. Wölcke, and G. B. Brown, *Tetrahedron*, **28**, 3 (1972).
(17) G. Stöhrer, G. Salemnick, and G. B. Brown, submitted.
(18) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N. Y., 1962.
(19) D. D. Perrin, *Aust. J. Chem.*, **16**, 572 (1963).
(20) J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, **36**, 2639 (1971).
(21) Although they are eluted close together from the column, the low absorption of 9-hydroxyxanthine and the very high absorption of 8-methylmercaptoxanthine at 290 nm permit a recognition of as little as 1% of the latter.


# 1,3-Bridged Aromatic Systems. XI. Stereochemistry of Reactions of Heterocyclic *N*-Oxides with Acetic Anhydride, Acetyl Chloride, and *p*-Toluenesulfonyl Chloride[1,2]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*Received April 10, 1974*


Reaction of the deuterium-labeled *N*-oxide **4** with acetic anhydride and acetyl chloride is highly stereospecific in that abstraction of syn deuterium leads to syn acetate **5**, while abstraction of anti hydrogen leads to anti acetate **6**; anhydro base **8**, in which the acetate function can rotate about the N–O axis, cannot, therefore, be an intermediate in these reactions. In reactions with acetyl chloride a bimolecular component of reaction diverts significant quantities of intermediate normally leading to anti acetate **6** to anti chloride **10**; the latter retains 95% of syn-deuterium label. Reaction of the unlabeled *N*-oxide of **4** with acetic anhydride-$^{18}O$ shows that both syn and anti acetates are formed both by intramolecular transfer of *N*-acetate to the benzylic carbon atom (78.8 and 73.9%, respectively) and by a process involving return of external acetate (21.2 and 26.1%, respectively). Distribution of $^{18}O$ label in both the CO and C=O functions of derived acetates has been determined.


The mechanism of reaction of alkylpyridine *N*-oxides with acid anhydrides (Scheme I) and acid halides has been studied in great detail, and reviewed.[3] In reactions leading to substitution at the $\alpha$-carbon atom it is generally thought that (1) step 1 is reversible,[4] (2) step 2 is generally rate determining,[4] (3) anhydro base (1) is an intermediate,[5] (4) step 3 involves heterolytic cleavage of the N–O bond (**2**),[6] and (5) step 4 is intramolecular in that it does not involve capture of external acetate.[4b,c,7]

The recent availability of syn-deuterium labeled **4**[2] has provided an opportunity to consider the stereochemical aspects of such reaction for the first time. The reaction of **4** with acetic anhydride is summarized in Scheme II.

Reaction of **4** with acetic anhydride gives syn and anti-acetates **5** and **6** in the ratio of 1:4 as compared to a ratio of 1:1.1 when unlabeled[8] **4** is employed. This isotope effect is consistent with previous observations that proton removal (step 2, Scheme I) is rate determining.[4] What was not anticipated was the stereospecificity observed; syn acetate **5** was formed almost exclusively (95%) by removal of deuterium, while anti acetate **6** was formed almost exclusively by removal of anti hydrogen (97%). Reaction of **4** with *p*-toluenesulfonyl chloride led exclusively to syn tosylate, as pre-

**Scheme I**