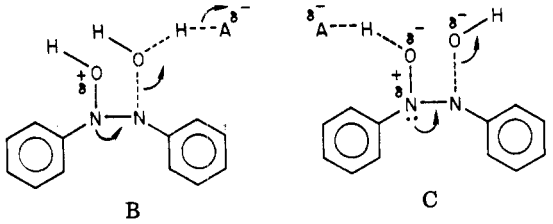

B C

tate-assisted removal of the proton from the symmetrical intermediate (as in C), increasing the electron density on the nitrogen to which the oxygen anion is bonded, facilitating electron-pair delocalization, and thereby catalyzing expulsion of hydroxide. The general catalysis rate constants determined in Figure 2 are actually apparent rate constants due to the rapid preequilibrium step shown in eq 1, i.e., $k_g = k_g'K_e$. Unfortunately, under these experimental conditions, the value of K_e is not determinable and thus neither are the actual values of k_{ga}' or k_{gb}'.

The establishment of general catalysis for the condensation of NOB and PHA provides further information regarding non-enzyme-controlled detoxication mechanisms for arylhydroxylamines at physiologically relevant pH's. Such catalysis thus offers an effective pathway for arylhydroxylamine depletion which is dependent upon catalytic groups available in an aqueous environment.

Experimental Section

NOB and AzB were obtained from Aldrich and recrystallized from methylene chloride–pentane prior to use. PHA was prepared by the method of Smissman and Corbett[11] and recrystallized from the same solvent prior to use.

Kinetic studies were carried out spectrophotometrically at 25 °C by monitoring the formation of AzB at 250 nm. The solutions were prepared by the addition of nitrogen-degassed metal-free[12] buffer (μ = 0.5, $NaClO_4$) to a volumetric flask containing an accurately weighed amount of NOB. After nitrogen was allowed to flow over the top of the solution and mixed, 3.0 mL was transferred to a cuvette containing PHA (concentration varied from 0.6 to 1.1 × 10^{-4} M after addition of NOB–buffer solution). Such reaction mixtures were prepared at a minimum of three NOB concentrations ($[NOB]_0$ = 1.0 to 2.1 × 10^{-3} M) at each of four buffer concentrations (0.01, 0.05, 0.25, and 0.50 M) at each of three pH's (4.92, 5.31, and 5.85). Reactions were followed for four to five half-lives under the established pseudo-first-order conditions. Values of k_{obsd} were obtained from plots of log ($OD_\infty - OD_t$) vs. time.

The rate constant for the oxidation of PHA in the absence of NOB, under the experimental conditions of the kinetic study, was determined in 0.01 and 0.50 M acetate buffer at pH 4.92 and 5.81. Product studies were performed by reverse-phase high-performance liquid chromatography on a system consisting of a Waters Model U6K injector, Model 6000 A pump, and Model 440 UV detector (Waters Associates) with an RP-18 column (Waters, μ Bondapak C_{18}). The mobile phase was 65:35 methanol–water at a flow rate of 2.0 mL/min. The concentration of AzB was monitored at 280 nm as a function of peak height on a strip chart recorder (V_R = 15 mL).

At the completion of the condensation reaction (determined by calculation from kinetic data) three high-performance LC determinations were made of the AzB concentration in each of four solutions (0.01 and 0.50 M acetate buffer at pH 4.92 and 5.81). The AzB was found to be stable in all solutions employed over the time period that reactions were monitored.

Acknowledgment. This work was supported by NIH Grant CA-19288 from the National Cancer Institute (PHS, DHEW).

Registry No. AzB, 495-48-7; NOB, 586-96-9; PHA, 100-65-2.

(11) E. E. Smissman and M. D. Corbett, *J. Org. Chem.*, **37**, 1847 (1972).
(12) R. E. Thiers, *Methods Biochem. Anal.*, **5**, 273–335 (1955).

Electrochemical Reduction of the Wieland-Miescher Ketone

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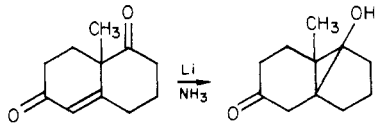
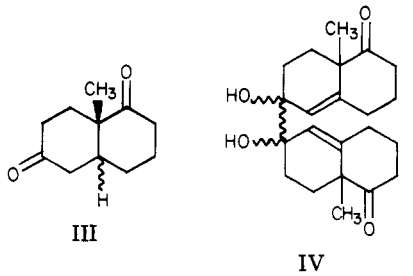
The chemistry of the reduction of the Wieland–Miescher ketone and some related compounds was reported in a series of papers by Reusch and co-workers.[1] In particular, this group was able to demonstrate that lithium/ammonia reduction resulted in a cyclopropanol derivative as the chief product, i.e., I to II. They also studied the polarography of this system and found that the half-wave reduction potentials of several enedione systems related to I were over 0.1 V more positive than the reduction potential of analogues lacking the isolated carbonyl group. This suggested very direct interaction between the enone moiety and the neighboring carbonyl group.[1]



I II

We wished to see if the polarography reported was reflected in the structure of the product of the electrochemical process, and, therefore, we undertook to effect a controlled-potential reduction of I which would allow product isolation. Toward this end, we repeated the polarography of I in 50% methanol/50% 0.1 N KCl and then carried out the controlled-potential reduction in the same medium. The crude product was chromatographically separated, and the chromatographic fractions were identified by using mass, ^1H NMR, and IR spectroscopy.

It was found that no cyclopropanol derivative II could be detected or isolated from the reduction. This material is easily distinguished from the products actually obtained, namely, III and IV, by means of its NMR spectrum.



III IV

Compound II, and similar substances, exhibits a resonance at about 1 ppm below Me_4Si, characteristic of a methyl substituting a cyclopropane ring.[1] The total crude reduction product showed no such resonance. There was recovered 85.4% product from the reduction (1.67 g of reactant yielding 1.43 g of product). Chromatography of this material gave a 79.3% recovery of fractions (1.14 g) consisting of a mixture of dihydro compounds, III (11%), and the two diastereomeric dimers of IV (89%). The mixture III was assigned structures on the basis of its IR (the presence of saturated carbonyls), NMR (no vinyl protons) and mass spectra (molecular ion at m/e 180). It

(1) W. Reusch, K. Grimm, J. E. Karoglan, J. Martin, K. P. Subrahamanian, Yock-Chai Toong, P. S. Venkatarsamani, J. D. Yordy, and P. Zoutendam, *J. Am. Chem. Soc.*, **99**, 1953 (1977); W. Reusch, K. Grim, J. E. Karoglan, J. Martin, K. P. Subrahamanian, P. S. Venkataramani, and J. D. Yordy, *ibid.*, **99**, 1958 (1977); J. D. Yordy and W. Reusch, *ibid.*, **99**, 1965 (1977).

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is possible that this minor product, III, could have arisen from a rearrangement of the cyclopropyl derivative II during the course of the electrochemical process; however, Reusch has shown the transformation to be effected by acid or base catalysis,[1] and our reduction conditions are neutral. The two diastereomers corresponding to IV were isolated as crystalline substances and their structures assigned on the basis of their IR (hydroxyl, carbon to carbon double bond, saturated carbonyl), NMR (vinyl proton), and mass spectra (no molecular ion but peaks at m/e 322, molecular ion less 2 mol of water, and m/e 179, dimer cleaved to monomer) and the C and H analysis.

It is thus apparent that the electrochemical reduction of I does not parallel the lithium/ammonia reduction. This result does not rule out the polarographic data as being indicative of a homoconjugative interaction as put forth by Reusch.[1]

Experimental Section

Analyses were performed by Atlantic Microlab, Inc. Infrared spectra were recorded on either a Perkin-Elmer Model 257 or a Model 467 spectrometer. NMR spectra were obtained on a Varian Model EM-360 spectrometer. Mass spectra were obtained on a Finnigan 4000 GC/MS system.

A Princeton Applied Research Model 170 was used for the controlled-potential electrolysis. The electrolysis cell was a conventional three-electrode system: a mercury (instrumental grade) pool working electrode (cathode), a saturated calomel reference electrode, and a silver auxiliary electrode (anode) which was separated from the solution by a fritted glass disk. The reduction was carried out under a nitrogen atmosphere. The mercury pool was stirred rapidly throughout the electrolysis with a magnetic stirrer. KCl was used as a supporting electrolyte. Prior to the electrolysis the system was purged with nitrogen for approximately 20 min until a steady background current for the solvent system, 1000 mL of 50:50 methanol–water, was obtained. The ketone (1.67 g, 0.009 mol), which had been dissolved in methanol, was added slowly to the solution. The electrolysis was conducted at -1.7 V vs. the SCE and 500 μA and was complete in 14 h, as indicated by a return to background levels of current. The methanol was rotoevaporated off and the aqueous solution extracted with ether. The ether layers were dried over Na_2SO_4 and concentrated to yield 1.43 g of yellow oil. This oil was chromatographed on 200 g of Silicar CC-7 with a gradient elution of hexanes/ether. Four main fractions were separated, the first two as oils (a total of 0.123 g) and then two as crystalline fractions (0.46 g and 0.51 g, respectively). Intermediate cuts between these two crystalline fractions contained 0.046 g of material.

The early-eluted materials showed carbonyl absorptions in the IR at 1700 cm^{-1}, were devoid of vinyl protons in the NMR, and exhibited no methyl resonances below 1.27 ppm. Their mass spectra contained a molecular ion peak at m/e 180. This evidence is consistent with these fractions being mixtures of the cis and trans isomers of III.

The major amount of product (89%) consisted of two crystalline fractions. In the IR the first of these (0.46 g, mp 163–164 °C) had a broad hydroxyl absorption (3580 cm^{-1}), a carbonyl absorption at 1700 cm^{-1}, and a double bond absorption at 1650 cm^{-1}. The NMR in $CDCl_3$ had a vinyl proton resonance at 5.87 ppm and no resonances below 1.23 ppm. The mass spectrum had peaks at m/e 322 (molecular weight of dimer less two H_2O's) and m/e 179 (half of the molecular weight). Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.71; H, 8.47.

In the IR the second crystalline product (0.51 g, mp 125–126 °C) had a broad hydroxyl absorption (3600 cm^{-1}), a carbonyl absorption at 1700 cm^{-1}, and a double bond absorption at 1650 cm^{-1}. The NMR in $CDCl_3$ had a vinyl proton resonance at 5.70 ppm and no resonances below 1.23 ppm. The mass spectrum had peaks at m/e 322 (molecular weight of dimer less two H_2O's) and m/e 179 (half of the molecular weight). Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.77; H, 8.46.

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Registry No. I, 20007-72-1; *cis*-III, 4707-05-5; *trans*-III, 4707-04-4; IV, 72952-32-0.

Use of (Pivaloyloxy)methyl as a Protecting Group in the Synthesis of Antigenic Theophylline (1,3-Dimethylxanthine) Derivatives†

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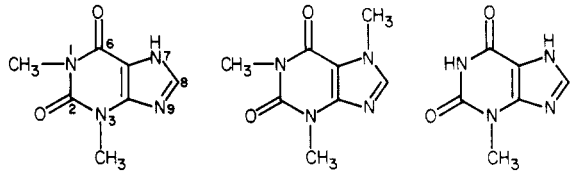
In the course of constructing a homogeneous enzyme immunoassay[1] for theophylline (1,3-dimethylxanthine, **1**)[2] in human serum, it became necessary to prepare an analogue of the drug that could be attached to an immunogenic protein carrier. Of primary concern is the design of such an analogue (hapten) which when attached to a carrier protein and injected into animals would afford antibodies to distinguish theophylline not only from its demethylated metabolites[3] but also from the ubiquitous presence of caffeine (1,3,7-trimethylxanthine, **2**). Since



1 **2** **3**

portions of the molecule that are least sterically encumbered by the protein carrier should most effectively interact with lymphocytic receptors, it seemed important to avoid attachment through the five-membered ring which bears the only structural feature that differentiates theophylline from caffeine. The choice between the remaining two sites of attachment to the drug at the 1- and 3-methyl groups was dictated in a similar way by the need to distinguish theophylline from 3-methylxanthine (**3**), a major metabolite.[3] For these reasons we wished to prepare 1-methyl-3-(carboxalkyl)xanthines in which the carboxyl group could serve as a linking function to ϵ-amino groups of the lysines of the protein carrier.[4]

Xanthines with identical alkyl substituents at the 1 and 3 positions are generally prepared from disubstituted ureas.[5] The use of unsymmetrical *N,N'*-dialkylureas[6] for the preparation of 1,3-dialkylxanthines would be expected to yield a mixture of products. Perhaps for this reason few unsymmetrical 1,3-dialkylxanthines have been reported. We wish to report a selective alkylation of 1-methylxanthine which makes 1-methyl-3-alkylxanthines[7] readily available.

Spectroscopic studies[8] indicate that the order of acidity of the N-bonded protons of xanthine is 3 > 7 > 1. This order suggests that controlled alkylation of 1-methylxanthine might give primarily 1-methyl-3-alkylxanthines. However only 1,7-dialkyl- and 1,3,7-trialkylxanthines have been observed under a variety of experimental conditions,[9] presumably because the higher rate of alkylation of the

† Contribution No 81.

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