Nonenzymatic Hydrolysis of Cocaine *via* Intramolecular Acid Catalysis

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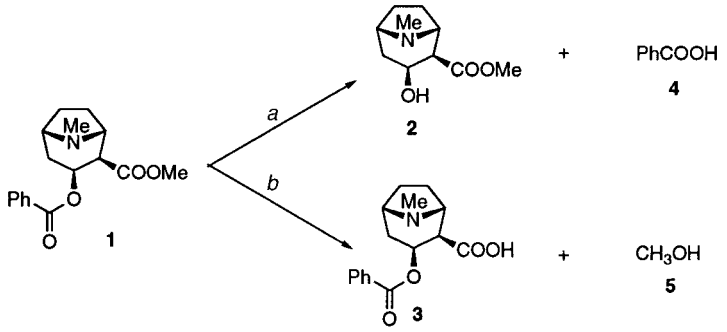
The spontaneous hydrolysis of the methyl-ester group of cocaine (**1**) *in vivo* contributes to the metabolic clearance of the drug in man. Neighboring-group participation by the tropane N-atom of cocaine in this hydrolysis was suggested by the normal stability of the methyl-ester groups of pseudococaine and *N*-acylnorcocaine. For cocaine, the relative rate of methyl-ester to benzoyl-ester hydrolysis was *ca.* 10:1 at pH $\leq$ 7.4, and, although absolute rates increased with increasing pH, their ratio collapsed at pH $>$ pK_a (8.6). These data are consistent with intramolecular acid catalysis of alkaline hydrolysis of the cocaine methyl-ester group under physiologic conditions.

The metabolic clearance of cocaine (*[1*R-*(*exo,exo*)]-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester*; **1**) *in vivo* is predominantly achieved by the hydrolysis of its benzoyloxy and methyl-ester moieties (*Scheme 1*). The benzoyl hydrolysis pathway (Path *a*), which converts cocaine to ecgonine methyl ester (**2**) and benzoic acid (**4**), is the better characterized and is found to require the mediation of hepatic or plasma esterases [1]. Methyl-ester hydrolysis, which yields *O*-benzoylecgonine (**3**) and methanol (**5**) (Path *b*) [2], can be catalyzed by a human liver carboxylesterase (hCE-1) [2a–c], but is also believed to occur spontaneously under physiological conditions (pH 7.4; 37°) [2d–e]. Some have attributed this putative lability of the methyl-ester moiety of cocaine (**1**) to alkaline factors or other artifact of sample handling [3]. Since the majority of commercially available methyl esters are quite stable under neutral conditions, it is fundamentally interesting to confirm and investigate the instability of the methyl-ester group of cocaine. Also, a particular interest in this hydrolysis stems from our ongoing investigation of antibody-catalyzed hydrolysis of cocaine at the benzoyloxy moiety as the basis for a new class of anti-addiction therapeutic agents [4]. An understanding of the mechanism of cocaine methyl-ester hydrolysis could guide the development of more effective transition-state analogs for the conversion of cocaine (**1**) to the biologically inactive product **2** [5]. Finally, the design of transition-state analogs for the antibody-catalyzed hydrolysis of the methyl-ester group of **1**, a process that is intrinsically more difficult due to the small size of the methyl-alcohol epitope, clearly requires an understanding of the mechanism for spontaneous hydrolysis.

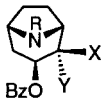
In this paper, investigations on the hydrolysis kinetics of the ester moieties of cocaine (**1**) under physiologic conditions and at a variety of pH values are reported. The close proximity of the methyl ester of cocaine to the tropane N-atom, and the

Scheme 1. *Cocaine Hydrolysis*



possibility of a proximity effect [6], led us to also study several related compounds in which either the substituents on the tropane N-atom were varied or the stereochemical relationship of the N-atom and the ester group was altered.

Sodium phosphate buffer (100 mM) with a specific pH value was mixed with cocaine (**1**) and its homologs **6**–**8**, respectively. Aliquots were analyzed by high-performance liquid chromatography (HPLC) immediately, and at intervals after incubation at 37°. HPLC peak integrals were measured to give the concentrations of substrate and



Cocaine (**1**) R = Me, X = CO_2Me, Y = H
Norcocaine (**6**) R = H, X = CO_2Me, Y = H
Pseudococaine (**7**) R = Me, X = H, Y = CO_2Me
N-Acetylnorcocaine (**8**) R = Ac, X = CO_2Me, Y = H

products and, the rate constant ratios (k_{met}/k_{ben}) of methyl-ester and benzoyloxy-group hydrolysis were then obtained. A linear relationship for $-\ln[Cs]$ *vs.* time indicated pseudo-first order kinetics for the hydrolysis of cocaine and its homologs (data not shown). Since k_{all} can be approximated as the sum of k_{met} and k_{ben} for the initial hydrolysis, the individual k_{met} and k_{ben} can be then calculated as shown in the *Table*.

Table. *Rate Constants of Ester Hydrolysis for Cocaine* (**1**) *and Homologs* **6**–**8** *at pH 7.4 and 37°*

Compound	k_{all} [h^{-1}][a])	$t_{1/2}$ [h]	k_{met} [h^{-1}][b])	k_{ben} [h^{-1}][c])	k_{met}/k_{ben}
1	0.0562	12.33	0.0509	0.00536	9.5
6	0.0176	39.29	0.0146	0.00306	4.8
7	0.00457	151.6	0.0216	0.00241	0.9
8	0.00108	641.8	0.00036	0.00072	0.5

[a]) Rate constant of overall hydrolysis. [b]) Rate constant of methyl-ester hydrolysis. [c]) Rate constant of benzoyloxy hydrolysis.

Cocaine (**1**) hydrolyzed significantly faster at pH 7.4 than its homologs **7** and **8**, and the difference was due primarily to the relatively high rate constant of the methyl-ester hydrolysis (k_{met}) (*Table*). Norcocaine (**6**), another tropane in which the N-atom and methoxycarboxyl group are proximate, also displayed a ratio of k_{met}/k_{ben} greater than 1. In contrast, pseudococaine (**7**), which possesses an equatorial methoxycarboxyl group,

and *N*-acetylnorcocaine (**8**) which possesses a nonbasic N-atom gave comparable k_{met}/k_{ben} ratios. These data are consistent with an intramolecular participation of the tropane N-atom, as an amine or ammonium salt, in the methyl-ester hydrolysis of cocaine (**1**). Unlike the hydrolytic behavior of *trans*- and *cis*-aryl 2-aminocyclohexanecarboxylates which showed little steric influence on the rates of hydrolysis [6e], the methyl-ester group of pseudococaine (**7**) was not approachable by the tropane N-atom due to a geometric constraint.

The rate constants for hydrolysis of the methyl-ester and benzoyloxy groups of cocaine (**1**) were determined (*Fig.*) and increased as pH was increased from 6.9 to 10.4, as expected for alkaline hydrolysis. At any given pH, the ratio k_{met}/k_{ben} exceeded 1, but the magnitude of this ratio declined toward 1 at pH values greater than the pK_a of **1** (8.6), suggesting that the ammonium salt of the tropane participated in the step determining k_{met}. In contrast, for pseudococaine (**7**), the magnitude of k_{met} equaled that of k_{ben} throughout the neutral to alkaline pH range for these reactions, and the proportionally increasing rates of hydrolysis with increasing pH are consistent with simple alkaline hydrolysis of these esters.

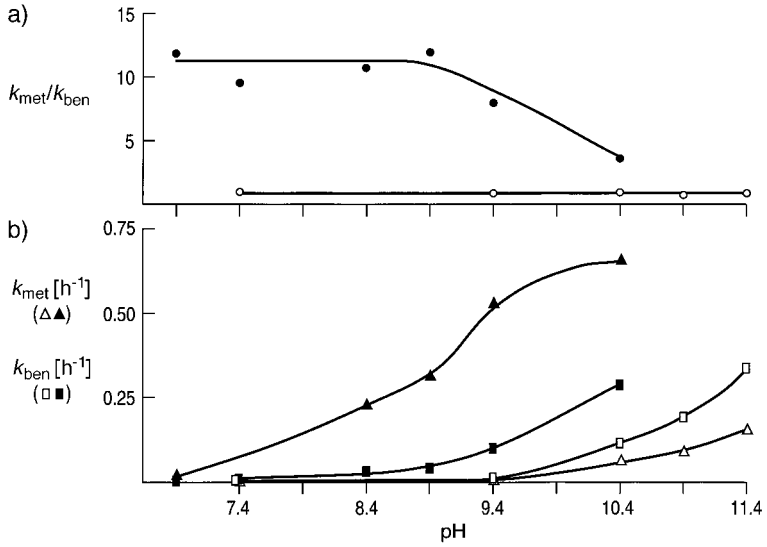
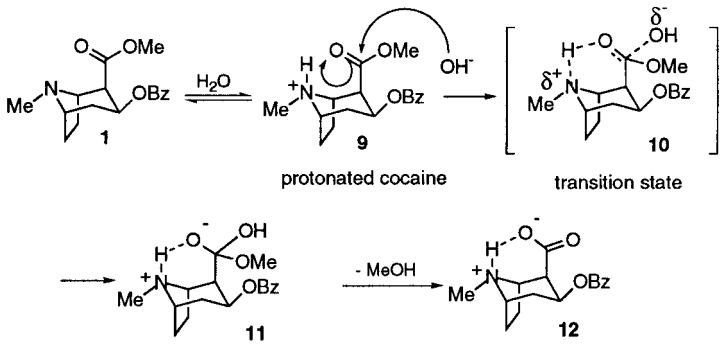


Figure. a) *pH Dependence of* k_{met}/k_{ben} *for cocaine* (**1**) (●) *and pseudococaine* (**7**) (○). b) *pH Dependence of* k_{met} (▲) *and* k_{ben} (■) *for cocaine* (**1**) *and* k_{met} (△) *and* k_{ben} (□) *for pseudococaine* (**7**). $I = 0.1$ M (NaCl).

Thus, the methyl-ester group of cocaine (**1**) is, indeed, much more labile than that of the benzoyloxy group under neutral conditions. The hydrolysis of the methyl-ester group of **1** can be assumed to involve intramolecular acid catalysis as shown in *Scheme 2*. This intramolecular catalysis would explain the relative lability of the methyl-ester group of cocaine (**1**) and norcocaine (**6**) compared to their benzoyloxy groups. The absence of such catalysis was also implied in the hydrolysis of the methyl-ester groups of pseudococaine (**7**) and *N*-acetylnorcocaine (**8**). This analysis is useful for the design of a new class of analogs to mimic the hypothesized transition state **10** for cocaine hydrolysis. We are now seeking to determine whether the monoclonal

Scheme 2. *A Rationale for the Rapid Hydrolysis of the Methyl-Ester Group of Cocaine* (**1**) *under Physiologic Conditions*



antibodies elicited by such analogs are catalytic [4][1]) and can promote the substrate-assisted degradation of cocaine.

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