loss of $C_7H_{10}O$ (*m/e* 438) and C_7H_5O (437) and base peaks corresponding to $C_7H_{11}O$ (111) and C_7H_5O (105), respectively. Except for the above-mentioned base peaks in the mass spectra of **1** and **2**, peaks in the region from *m/e* 438 to 69 were almost identical with those present in the mass spectrum of bruceine B (**3**). Inspection of the nmr spectra of bruceantin (**1**), bruceantarin (**2**), and bruceine B (**3**) revealed that all three displayed peaks corresponding to an angular methyl group in the region of τ 8.3–8.6, a vinyl methyl at 8.0–8.2, a methoxyl at 6.2–6.5, and a sharp one-proton doublet (J = 13 Hz) between 3.2 and 3.6 [assigned to H-15 in bruceine B (**3**)[8]]. The major differences between the nmr spectra of bruceantin (**1**) and bruceine B (**3**) were the additional signals for **1** of a six-proton doublet (J = 6.5 Hz) at τ 8.88, a vinyl methyl signal at 7.82, and a vinyl proton singlet at 4.39. These data and the presence of the base peak at *m/e* 111 in the mass spectrum supported formulation of bruceantin (**1**) as the 3,4-dimethylpent-2-enoic acid ester of bruceolide[8] (**4**). Hydrogenation of bruceantin (**1**) gave dihydrobruceantin (**5**): $C_{28}H_{38}O_{11}$; mp 137–140°; $[\alpha]^{25}D$ −64.5° (*c* 2.9, pyridine); mass spectrum *m/e* 550 (M^+), 438, 297, 151, and 113. That only the side-chain ester of **1** had been reduced was indicated by the uv spectrum, which still showed the diosphenol absorption and alkaline shift, and by the nmr spectrum, which showed no olefinic proton but a new three-proton doublet (J = 6.5 Hz) at τ 9.06. Mild alkaline hydrolysis of **5** gave bruceolide (**4**). In addition, alkaline hydrolysis of bruceantin (**1**) and esterification of the steam-distillable acid with diazoethane gave ethyl *trans*-3,4-dimethyl-2-pentenoate.[9] In the nmr spectrum of ethyl *cis*-3,4-dimethyl-2-pentenoate the vinyl methyl signal appeared at τ 8.25, whereas the corresponding peak for the trans isomer occurred at 7.90. The peak attributed to the ester vinyl methyl in **1** appeared at τ 7.82, indicative of trans stereochemistry in bruceantin (**1**).

The sharp one-proton doublet at τ 3.79 (J = 13 Hz) in the nmr spectrum of **1** indicated C-15 as the point of attachment of the ester side chain. The corresponding peak in the spectrum of dihydrobruceantin (**5**) appeared at τ 3.14 (J = 13 Hz) and in that of bruceine B (**3**) at τ 3.28 (J = 13 Hz).

In the nmr spectrum of bruceantarin (**2**), a complex A_2B_2X system centered at τ 2.3 was indicative of the presence of a benzoate group. In addition, the sharp one-proton doublet (J = 13 Hz) at τ 3.58 and the base peak at *m/e* 105 in the mass spectrum supported for bruceantarin (**2**) the C-15 benzoate ester structure. The postulated structure was confirmed by mild alkaline hydrolysis of bruceantarin (**2**) to benzoic acid and bruceolide (**4**).

The observed potent antileukemic activity of bruceantin confirms and extends an earlier report of antitumor activity of a simaroubolide.[10] The markedly higher potency of bruceantin (**1**),[7] compared with that of bruceantarin (**2**) and bruceine B (**3**), may be attributable to the role of the α,β-unsaturated ester.[11] Investigations are in progress to determine the significance of the unsaturated ester, the diosphenol, and of other structural features in relation to the tumor-inhibitory activity of bruceantin.

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Intramolecular Electrostatic Stabilization of an SNl Transition State

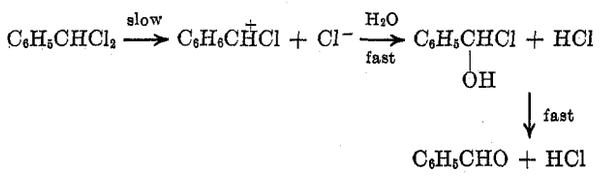
Summary: *o*-Carboxybenzal chloride hydrolyzes at about the same rate as the para isomer in water but 110 times as fast as in 60% aqueous dioxane.

Sir: The mechanism proposed for the hydrolytic action of lysozyme[1] involves at least two essential features. The first of these is the suggestion that Glu-35 acts as a general acid[2] effecting intracomplex protonation of the acetal linkage. It seems to be well established that a carboxyl group can function as an intermolecular general acid in acetal hydrolysis.[3] Intramolecular general acid catalysis has also recently been observed in the hydrolysis of 2-(*o*-carboxyphenoxy)tetrahydropyran in aqueous dioxane.[4] The second feature of the proposed enzymatic mechanism is that the ionized form of Asp-52 functions either as a nucleophile forming a glycosyl enzyme intermediate or electrostatically stabilizes the transition state leading to the oxocarbonium ion intermediate. In a very careful study Dunn and Bruice[5] have provided evidence that an ortho carboxylate ion can electrostatically stabilize the transition state in the A-1 cleavage of acetals and that this type of stabilization can provide substantial rate enhancements. To obtain additional information concerning the role of an ionized carboxyl group in stabilizing an ionic transition state uncomplicated by a proton-transfer step we have studied the hydrolysis of *o*- and *p*-carboxybenzal chlorides.

The mechanism of hydrolysis of benzal chlorides has been the subject of numerous reports.[6] It is clear that the mechanism involves rate-determining formation of a chlorocarbonium ion followed by a series of rapid steps leading to the product aldehyde (Scheme I). For example, ρ^+ calculated from published[6a] data is -5.2 ± 0.3. Also the rate is completely unaffected by external nucleophiles[6b,c,e] and the value

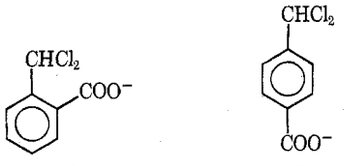
(1) Reviews: P. Jolles, *Angew. Chem., Int. Ed. Eng.*, **3**, 28 (1964); P. Jolles, *ibid.*, **8**, (1969); B. Capon, *Chem. Rev.*, **69**, 434 (1969); (a) R. E. Canfield, *J. Biol. Chem.*, **238**, 2699 (1963); (b) C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Nature (London)*, **206**, 757 (1965); (c) L. N. Johnson and D. C. Phillips, *ibid.*, **206**, 761 (1965); (d) J. B. Howard and A. N. Glazier, *J. Biol. Chem.*, **244**, 1399 (1969); (e) J. A. Rupley, *Proc. Roy. Soc. Ser. B. (London)*, **167**, 416 (1967); (f) D. C. Phillips, *Sci. Amer.*, **215**, 78 (1966); (g) J. A. Rupley and V. Gates, *Proc. Nat. Acad. Sci. U. S.*, **57**, 496 (1967); (h) T. Osawa and Y. Nakasawa, *Biochem. Biophys. Acta*, **130**, 56 (1966); (i) M. A. Raftery and T. Rand-Meir, *Biochemistry*, **7**, 3281 (1968).
(2) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc. B (London)*, **167**, 378 (1967).
(3) T. H. Fife, *Accounts Chem. Res.*, **5**, 264 (1972).
(4) T. H. Fife and E. Anderson, *J. Amer. Chem. Soc.*, **93**, 6610 (1971).
(5) B. M. Dunn and T. C. Bruice, *ibid.*, **93**, 5725 (1971).
(6) (a) F. Asinger and G. Lock, *Monatsh. Chem.*, **62**, 323 (1933); (b) S. C. T. Olivier and A. Weber, *Recl. Trav. Chim. Pays-Bas*, **53**, 869 (1934); (c) L. J. Andrews and W. W. Kaeding, *J. Amer. Chem. Soc.*, **73**, 1007 (1951); (d) B. Bensley and G. Kohnstam, *J. Chem. Soc.*, 287 (1956); (e) K. Tanabe and T. Ido, *J. Res. Inst. Catal., Hokkaido Univ.*, **12**, 223 (1965).

SCHEME I



of m measured in aqueous ethanol solutions for benzal chloride is large (1.31 ± 0.02)[7] indicative of an SN1 transition state.[8]

We have measured the hydrolysis rates of *o*-carboxybenzal chloride (1) and *p*-carboxybenzal chloride (2) in water and mixtures of water and dioxane in the



1

2

presence of excess base and these results are collected in Table I. These results show that in water an ortho

TABLE I

RATES OF HYDROLYSIS OF CARBOXY SUBSTITUTED BENZAL CHLORIDES AT 25.2 ± 0.2° [a]

$10^7 k_{ortho}^{obsd}$, sec^{-1}	$10^7 k_{para}^{obsd}$, sec^{-1}	k_{ortho}/k_{para}	Solvent[b]
2210	2640	0.83	A
563	11.2	50.3	B
276	2.52	110	C

[a] Rate constants were determined by following the increase of absorbance at 257 nm due to the aldehyde product. The slower reactions were studied using an initial rate method and the rate constants thus obtained are considered accurate to ±10%. The results are the average of at least three separate determinations. [b] A, 0.2 *N* NaOH in water; B, 50% dioxane–50% 0.2 *N* NaOH (v/v); C, 60% dioxane–40% 0.2 *N* NaOH (v/v).

carboxylate ion does *not* facilitate the reaction and, in fact, has a slight rate-retarding effect. Presumably in water (a highly polar solvent) the ortho carboxylate ion does not compete effectively with the solvent in stabilizing the ionic transition state. However, increasing the amount of dioxane present in the solvent results in a dramatic increase in k_{ortho}/k_{para} (Table I). Thus, as the solvent becomes less able to stabilize the transition state electrostatic stabilization by the ortho carboxylate ion becomes more pronounced. In fact, extrapolation of the data in Table I give k_{ortho}/k_{para} = 7600 in 90% aqueous dioxane.

An alternative mechanism involving intramolecular nucleophilic displacement by the ortho carboxylate function (Scheme II) cannot rigorously be excluded at the present time.[9]

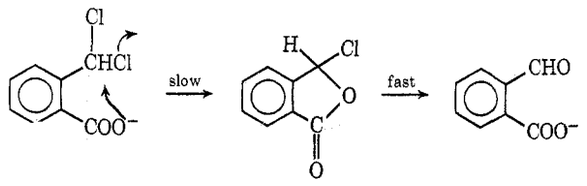
However, if intramolecular displacement was responsible for the high k_{ortho}/k_{para} values in aqueous dioxane solutions, one would expect an even large value of k_{ortho}/k_{para} in aqueous DMSO (*cf.* the large

(7) N. Grossman, unpublished work.

(8) A. Streitweiser, Jr., "Solvolytic Displacement Reactions," Vol. X, McGraw-Hill, New York, N. Y., 1962, pp 45–47.

(9) An additional mechanism suggested by a reviewer involving the intramolecular deprotonation of the dichloromethyl group followed by α elimination to generate a carbene seems unlikely in view of the fact that the rate is independent of the concentration of HO^- (a much stronger base) for both **1** and **2** if pH > pK_A.

SCHEME II



rate accelerations observed[10] for SN2 reactions in DMSO and the increased rate of anhydride formation from phenyl hydrogen phthalate in aqueous DMSO).[11] On the other hand, electrostatic catalysis ought to be favored in media of low dielectric constant and, since DMSO has a higher dielectric constant (~50) than dioxane (~2), a lower value of k_{ortho}/k_{para} is expected in aqueous DMSO. Since k_{ortho}/k_{para} = 12 in 50% aqueous DMSO it would seem that the ortho carboxylate ion enhances the solvolysis rate of benzal chloride by electrostatically stabilizing the ionic transition state rather than effecting an intramolecular nucleophilic displacement.

In conclusion, we feel that the results described in the communication support the suggestion that, under certain conditions, a properly oriented carboxylate ion can stabilize a transition state leading to a resonance stabilized carbonium ion.

Acknowledgment.—We are grateful to the National Science Foundation for financial support of this work (Grant No. GP-29738 X).

(10) J. J. Delpuech, *Bull. Soc. Chim. Fr.*, 1624 (1966).

(11) T. C. Bruice and A. Turner, *J. Amer. Chem. Soc.*, **92**, 3422 (1970).

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A Reinvestigation of 3,5′-Anhydro-2′,3′-*O*-isopropylideneinosine

Summary: Nmr studies have shown that 3,5′-anhydro-2′,3′-*O*-isopropylideneinosine (II), previously described, is actually the ring-opened compound 5′,N^5-anhydro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-formamidoimidazole-4-carboxamide (I); the preparation and characterization of authentic II is described.

Sir: The formation of cyclonucleosides from purine nucleoside derivatives is well established.[1] The first reported synthesis[2] of 3,5′-anhydro-2′,3′-*O*-isopropylideneinosine used thermal cyclization of the appropriate 5′-*O*-*p*-toluenesulfonyl derivative in an inert solvent, a procedure first used in the synthesis of 3,5′-anhydro-2′,3′-*O*-isopropylideneadenosine.[3] The inosine cyclonucleoside was isolated as the *p*-toluenesulfonate salt[2] which was converted to a product described as the monohydrate of 3,5′-anhydro-2′,3′-*O*-isopropylideneinosine. Three subsequent reports have described the formation of the same final product by the following

(1) C. A. Dekker and L. Goodman in "The Carbohydrates," Vol. IIA, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, New York, N. Y., 1970, Chapter 29.

(2) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963).

(3) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).