Chemical Model for a Mechanism of Inactivation of Monoamine Oxidase by Heterocyclic Compounds. Electronic Effects on Acetal Hydrolysis

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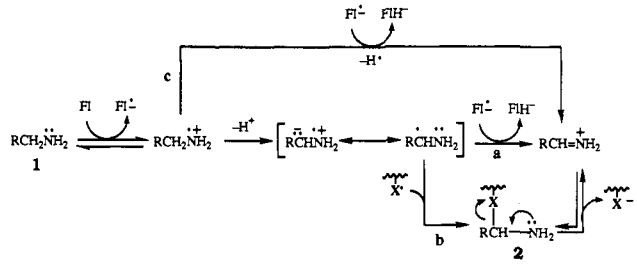
Abstract: Monoamine oxidase (MAO) was shown previously to undergo time-dependent inhibition by 5-(aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (**3**, X = N, Y = O, R = Me, R′ = H), *cis*- and *trans*-5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**5**, R = Me), and 4-(aminomethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone (**6**, R = Me). Two approaches are taken in this article to test the hypothesis that the cause for this inhibition is electron-withdrawing stabilization of an enzyme adduct by the heterocycles. First, the rates of reactivation of the inhibited enzyme were measured, and they correlated qualitatively with the strengths of the electron-withdrawing abilities of the heterocycles. The second approach was a chemical model study for the proposed enzyme adduct stabilities. The corresponding acetals were synthesized, and the rates of acid hydrolysis of these acetals were used as a model for the decomposition of the enzyme adducts; the rates of hydrolysis should be a qualitative measure of the stabilities of the enzyme adducts. An inverse relationship was observed between the strength of the electron-withdrawing effect of the heterocycle and the rate of acetal hydrolysis. These results support the hypothesis that inhibition of MAO by heterocyclic compounds results from electronic stabilization of the enzyme adducts produced. This realization should prove to be very beneficial to the design of new classes of MAO inhibitors. Furthermore, it cautions synthetic chemists as to the problems associated with acetal deprotection of aldehydes when electron-withdrawing groups are even three bonds away from the acetal carbon.

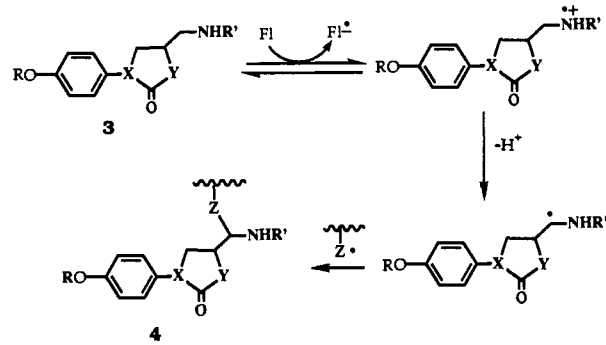
Introduction

Monoamine oxidase (EC 1.4.3.4; MAO) is an important flavoenzyme in the degradation of a variety of amine neurotransmitters.[1] It exists in two principal isozymic forms known as MAO A and MAO B.[2] Compounds that inhibit MAO A exhibit antidepressant effects,[3] and those that inhibit MAO B are useful as adjuncts in the treatment of Parkinson's disease.[4] A family of mechanisms for MAO-catalyzed amine oxidation that involves radical intermediates (Scheme I) has been proposed.[5] 5-(Aminomethyl)-3-aryl-2-oxazolidinones have been shown to be potent and selective MAO B inactivators.[6] The mechanism for inactivation of MAO B by 5-(aminomethyl)-3-aryl-2-oxazolidinones (**3**, X = N, Y = O; Scheme II) was investigated by treatment of the isolated enzyme with analogues containing radioactive labels in the aryl substituent and at the oxazolidinone carbonyl carbon atom.[7] It was found that both the aryl moiety and the oxazolidinone ring remain intact during inactivation and become attached to the enzyme. This observation was rationalized[7] by a mechanism very similar to that proposed for amine oxidation which involves single-electron transfer from the amine to the flavin followed by loss of a proton and either radical coupling to an active site radical (Scheme II; X = N, Y = O) or a second electron transfer followed by nucleophilic addition to the resulting iminium ion. The only difference in the mechanism for the oxidation of substrates to give products (Scheme I, pathway b) and the oxidation of the 5-(aminomethyl)-3-aryl-2-oxazolidinones leading to inactivation (Scheme II; X = N, Y = O) is the stability of the hypothetical intermediates (**2** vs **4**) that are formed. It was suggested that the 5-(aminomethyl)-3-aryl-2-oxazolidinones are really just substrates for MAO, but inactivation arises because the covalent intermediate (**4**) is much more stable than that for normal substrates of the enzyme (**2**). A hypothesis that was offered[7] for the stability of the inactivator adduct was that the

Scheme I



Scheme II



(1) Strolin Benedetti, M.; Dostert, P.; Tipton, K. F. *Prog. Drug Metab.* **1988**, *11*, 149–174.
(2) Johnston, J. P. *Biochem. Pharmacol.* **1968**, *17*, 1285–1297.
(3) Ives, J. L.; Heym, J. *Annu. Rep. Med. Chem.* **1989**, *24*, 21–29.
(4) Palfreyman, M. G.; McDonald, I. A.; Bey, P.; Schechter, P. J.; Sjoerdsma, A. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1988**, *12*, 967–987.
(5) Banik, G. M.; Silverman, R. B. *J. Am. Chem. Soc.* **1990**, *112*, 4499–4507.
(6) (a) Dostert, P.; Strolin Benedetti, M.; Jalfre, M. In *Monoamine Oxidase: Basic and Clinical Frontiers*; Kamujo, K., Ed.; Excerpta Medica: Amsterdam, 1982; pp 155–163. (b) Ancher, J. F. *Drugs Future* **1984**, *9*, 585–586. (c) Tipton, K. F.; Fowler, C. J.; McCrodden, J.; Strolin Benedetti, M. *Biochem. J.* **1983**, *209*, 235–242.
(7) Gates, K. S.; Silverman, R. B. *J. Am. Chem. Soc.* **1990**, *112*, 9364–9372.

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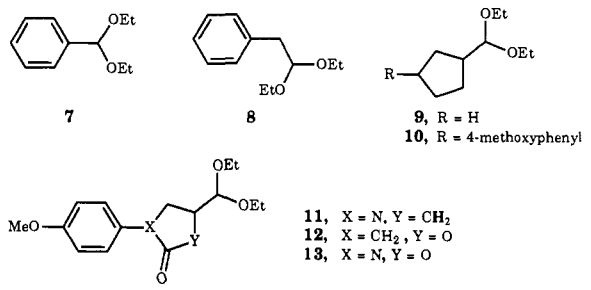
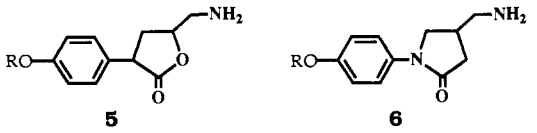


Figure 1. Diethyl acetals investigated.

amino heteroatom acetal adduct (**4**, Z = amino acid heteroatom) was stabilized by the electron-withdrawing ability of the heteroatoms in the oxazolidinone ring.

On the basis of this electron-withdrawing hypothesis for adduct stability, two new classes of inactivators of MAO were designed: the 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones **5**[8] and the 4-(aminomethyl)-1-aryl-2-pyrrolidinones **6**.[9] In the case of the



dihydrofuranone analogues **5**, it is not surprising that the oxygen atom in the ring should have the dominant electron-withdrawing effect and that compounds **5** could give adducts with stabilities comparable to those produced by the oxazolidinones (**3**; X = N, Y = O). However, because inductive effects are distance dependent, it was somewhat unexpected that the corresponding pyrrolidinones **6**, which have an electron-withdrawing nitrogen three atoms away from the site of attachment to the enzyme, were inactivators of MAO. If the electron-withdrawing hypothesis is relevant to the inactivation process, then the stabilities of the various enzyme adducts should be a function of the strength of the inductive effect. Here we show that the adduct stabilities for three classes of heterocyclic compounds correlate with the electron-withdrawing effects and that a chemical model for these effects, namely, acetal hydrolysis, supports this hypothesis.

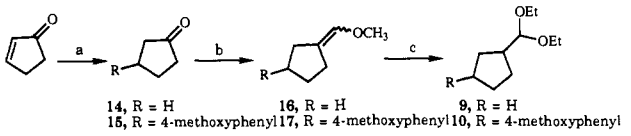
Results

Stabilities of the Adducts Formed by Inactivation of MAO with Heterocyclic Compounds. MAO was incubated with 5-(aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (**3**, X = N, Y = O, R = Me, R′ = H), *cis*- and *trans*-5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**5**, R = Me), and 4-(aminomethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone (**6**, R = Me) until no enzyme activity remained. Each was dialyzed against 50 mM potassium phosphate (pH 7.4), and the rate of return of enzyme activity was found to be pseudo-first-order. The half-lives for reactivation of the oxazolidinone **3**, the *cis*- and *trans*-dihydrofuranones **5**, and the pyrrolidinone 6 were 160, 115, 130, and 22 min, respectively. The stabilities of these adducts correlate with the expected electron-withdrawing effects of the heteroatoms; the nitrogen atom in **6** (R = Me) is three atoms away from the proposed site of attachment to the enzyme and the oxygen atom in **5** (R = Me) is two atoms away from the attachment site, and these effects should be additive in **3** (X = N, Y = O, R = Me, R′ = H).

Syntheses of Model Acetals. The diethyl acetals that were synthesized for the model study are shown in Figure 1.

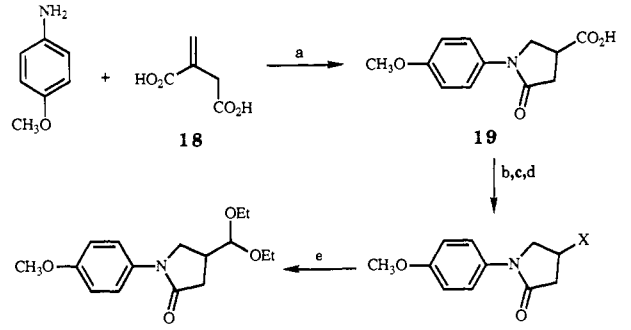
(A) Diethyl Acetals of Benzaldehyde 7 and Phenylacetaldehyde 8. The procedure of Sugai et al.,[10] treatment of the aldehydes with triethyl orthoformate in ethanol and a catalytic amount of *p*-toluenesulfonic acid, was used.

Scheme III[a]



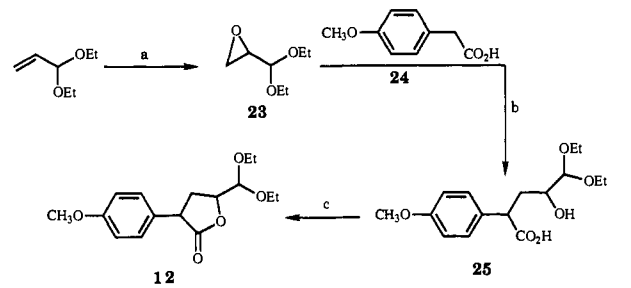
[a] (a) 4-Bromoanisole, *n*-BuLi, Cu_2Br_2, ether; (b) $CH_3OCH_2PPh_3Cl$, *t*-BuOK, THF; (c) $HC(OEt)_3$, *p*-TsOH, EtOH.

Scheme IV[a]



[a] (a) Δ, H_2O; (b) $SOCl_2$, CH_3OH, 0 °C; (c) $NaBH_4$, CH_3OH, Δ; (d) PCC, 0 °C; (e) $HC(OEt)_3$, *p*-TsOH, EtOH.

Scheme V[a]



[a] (a) Benzonitrile, H_2O_2, CH_3OH; (b) *n*-BuLi, THF; (c) benzene, *p*-TsOH, Δ.

(B) Diethyl Acetals of Cyclopentylformaldehyde 9 and [3-(4-Methoxyphenyl)cyclopentyl]formaldehyde 10. The synthetic route to these compounds is shown in Scheme III. Conjugate addition of the organocuprate prepared from 4-bromoanisole to 2-cyclopentenone[11] gave 3-(4-methoxyphenyl)cyclopentanone (**15**). The methyl vinyl ethers **16** and **17** were synthesized by Wittig reactions of the cyclopentanones **14** and **15**, respectively, with the ylide of (methoxymethyl)triphenylphosphonium chloride.[11] Treatment of **16** and **17** with triethyl orthoformate in ethanol and a catalytic amount of *p*-toluenesulfonic acid gave **9** and **10**, respectively.

(C) Diethyl Acetal of 4-Formyl-1-(4-methoxyphenyl)-2-pyrrolidinone 11. As shown in Scheme IV, condensation of anisidine with itaconic acid (**18**) followed by cyclization gave the carboxylic acid **19**, which was esterified to **20**, reduced to **21**,[9] and oxidized to the corresponding aldehyde **22**.[12] Conversion to the diethyl acetal as described above gave **11**.

(D) Diethyl Acetal of 5-Formyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one 12. Condensation of the dilithium salt of (4-methoxyphenyl)acetic acid (**24**, Scheme V) with glycidaldehyde diethyl acetal (**23**), prepared from acrolein diethyl acetal,[13] gave

(8) Ding, Z.; Silverman, R. B. *J. Med. Chem.* **1992**, *35*, 885–889.
(9) Ding, C. Z.; Silverman, R. B. *J. Enzyme Inhib.* **1992**, *6*, 223–231.
(10) Sugai, S.; Kodama, T.; Akaboshi, S.; Ikegami, S. *Chem. Pharm. Bull.* **1984**, *32*, 99–105.
(11) Ye, Q.; Grunewald, G. L. *J. Med. Chem.* **1989**, *32*, 478–486.
(12) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.
(13) Durrwachter, J. R.; Drueckhammer, D. G.; Nazaki, K.; Sweers, H. W.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812–7818.

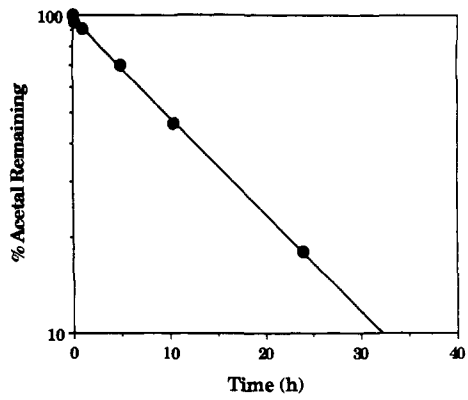
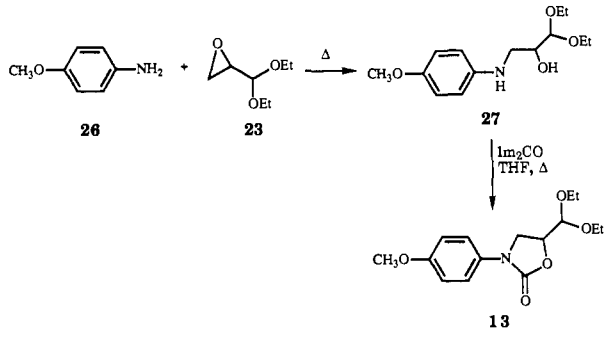


Figure 2. Rate of hydrolysis of acetal **11**. See the Experimental Section for the procedure.

Scheme VI



the γ-hydroxy acid **25**,[14] which was lactonized under Dean–Stark conditions to give a mixture of two diastereomeric lactone acetals **12**.

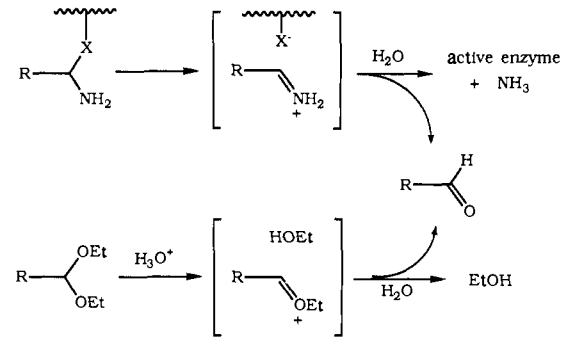
(E) Diethyl Acetal of 5-Formyl-3-(4-methoxyphenyl)-2-oxazolidinone 13. Alkylation[15] of anisidine (**26**, Scheme VI) with **23** produced the amino alcohol **27**, which was converted into the oxazolidinone **13** with carbonyldiimidazole.[16]

Hydrolysis of the Acetals. The rates of hydrolysis of the various acetals were measured in a mixture of two parts acetone-d_6 and one part D_2O containing 0.5% DCl. Figure 2 gives a typical plot of the results (in this case, for **11**). All of the results are summarized in Table I. The products of hydrolysis of acetals **7–10** are the corresponding aldehydes, but the products of acetals **11–13** are the corresponding hydrates (by NMR).

Discussion

MAO undergoes time-dependent inhibition by three heterocyclic compounds: a pyrrolidinone (**6**, R = Me),[9] a dihydrofuranone (**5**, R = Me),[8] and an oxazolidinone (**3**, X = N, Y = O, R = Me, R′ = H).[6,7] We proposed earlier[7-9] that the inhibition resulted from the formation of a covalent adduct of the inhibitors with an active site residue of MAO. The relative stability of this adduct was proposed to be the result of the electron-withdrawing ability of the heteroatoms in the heterocyclic rings. In order to test this hypothesis, two approaches were taken. First, the relative stabilities of the adducts formed by time-dependent inhibition of MAO with the three heterocyclic compounds were determined by measuring the rates of reactivation of the enzyme. The greater the electron-withdrawing effect of the heterocycle, the greater the stability of the adduct should be, and this should translate into a greater half-life for reactivation. The rates of reactivation correspond to the electron-withdrawing effects of the heteroatoms in the rings: the oxazolidinone stability (two heteroatoms) > the lactone stability (oxygen two atoms from the site of enzyme attachment) > the pyrrolidinone stability (nitrogen three atoms from the site of enzyme attachment). The rationale for why the increased electron-withdrawing ability increases the stability of the enzyme adduct is that it stabilizes the sp^3 hybridization of the carbon atom involved in the covalent adduct formation with the enzyme residue (e.g., the α-carbon of **4** in Scheme II). Also, the electron-withdrawing effect destabilizes the sp^2 hybridization that would form upon cleavage of the enzyme–inhibitor bond, leading to the iminium ion.

Scheme VII



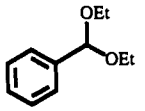
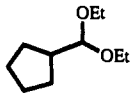
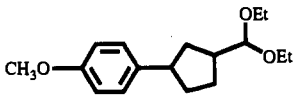
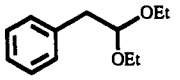
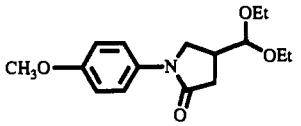
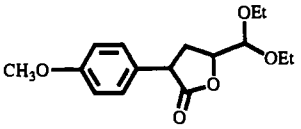
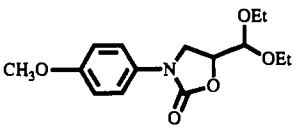
The second approach taken to test the electronic effect hypothesis was a chemical model study for the proposed stabilities of enzyme adducts (**4**, Scheme II). It was thought that acid hydrolysis of the corresponding acetals (Scheme VII) should be a reasonable model for the decomposition of the enzyme adducts and that the rates of hydrolysis should be a qualitative measure of the stabilities of **2** (Scheme I) and **4** (Scheme II) or, at least, of the inductive effect of the R group on acetal protonation and decomposition. If this hypothesis is correct, then the hydrolysis of the acetal of a normal substrate for MAO should proceed very rapidly, whereas the corresponding acetals of the oxidation products of the heterocyclic inhibitors should hydrolyze slowly; the relative rates of hydrolysis should be inversely proportional to the electron-withdrawing effect. Since the fast step in acetal hydrolysis is generally protonation, presumably the effect of the electron-withdrawing groups on the acetal protonation equilibrium would not be important relative to the effect on the rate-determining C–O bond cleavage step. Both of these effects, however, are in the same direction, so that it may not matter which step or steps are affected. In carbohydrate chemistry the effect of electron-withdrawing groups adjacent to the anomeric carbon of β-glucosides on their acid-catalyzed hydrolyses has been known for over 25 years.[17]

The diethyl acetals of benzaldehyde and phenylacetaldehyde, models for adduct **2** (Scheme I) where R = Ph and R = $PhCH_2$, mimic the adducts formed if benzylamine and phenethylamine, respectively, very good substrates for MAO, are the substrates. The diethyl acetals of 4-formyl-1-(4-methoxyphenyl)-2-pyrrolidinone **11**, 5-formyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one **12**, and 5-formyl-3-(4-methoxyphenyl)-2-oxazolidinone **13** are models for the proposed adducts formed between MAO and 4-aminomethyl-1-(4-methoxyphenyl)-2-pyrrolidinone (**6**, R = Me), 5-aminomethyl-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**5**, R = Me), and 5-(aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (**3**, X = N, Y = O, R = Me, R′ = H), respectively. The results of the acid hydrolysis study are summarized in Table I. It is apparent that electron-withdrawing ability has a substantial effect on the stability of the acetals, and by analogy, the stability of the corresponding enzyme intermediate adducts.

The rate of hydrolysis of the acetal of benzaldehyde, a model for the benzylamine adduct, is the fastest because ionization leads to a resonance-stabilized carbenium ion. In the case of the acetal of phenylacetaldehyde, the phenyl group can only destabilize the

(14) Creger, P. L. *J. Am. Chem. Soc.* **1967**, *89*, 2500–2501.
(15) Gregory, W. A.; Britteli, D. R.; Wang, C.-L. J.; Wuonola, M. A.; McRipley, R. J.; Eustice, D. A.; Eberly, V. S.; Bartholomew, P. T.; Slee, A. W.; Forbes, M. *J. Med. Chem.* **1989**, *32*, 1673–1681.
(16) Staab, H. A. *Angew. Chem., Int. Ed. Engl.* **1962**, *1*, 351–367.
(17) Capon, B. *Chem. Rev.* **1969**, *69*, 407–498.

Table I. Rates of Acid Hydrolysis of Various Diethyl Acetals[a]

acetal	$t_{1/2}$	k (min^{-1})	k_{rel}
	0.47 s[b]	83.5	4.2×10^2
	2.0 min	0.35	1.75
	3.5 min	0.2	1.0
	26 min	0.027	0.135
	9.5 h	1.2×10^{-3}	6×10^{-3}
	18.75 days	2.6×10^{-5}	1.3×10^{-4}
	1.6 yr[c]	8.3×10^{-7}	4.2×10^{-6}

[a] Two parts acetone-d_6 and one part D_2O containing 0.5% DCl at 23 °C. [b] Benzaldehyde diethyl acetal hydrolyzed instantaneously under the conditions used. The half-life, therefore, was calculated as follows: benzaldehyde diethyl acetal was hydrolyzed in a mixture of acetone-d_6 and D_2O in the presence of a catalytic amount of p-TsOH; the half-life was 16 s. Under the same conditions, the half-life for hydrolysis of the diethyl acetal of 1-formyl-3-(4-methoxyphenyl)cyclopentane **10** was 120 min. The rate for benzaldehyde diethyl acetal hydrolysis under the conditions used in this table, therefore, was determined by extrapolation from the rate for **10** under these conditions on the basis of the ratio of the hydrolysis rates for these compounds with catalytic p-TsOH hydrolysis. [c] This value is based on an extrapolation from only 20% hydrolyzed.

incipient carbenium ion by an inductive electron-withdrawing effect. Therefore, this acetal hydrolyzes much more slowly than the acetal of benzaldehyde. This electron-withdrawing effect, however, is minor relative to the electron-withdrawing effects of the pyrrolidinone **11**, lactone **12**, and oxazolidinone **13** rings, which stabilize the acetals much more strongly. Furthermore, the stabilizing effect is in the same order as that observed for the enzyme adduct stabilities produced by the three corresponding heterocyclic inhibitors. The diminution of hydrolysis rates with the heterocyclic compounds is not the result of the cyclic structure, because the corresponding cyclopentane, namely, the diethyl acetal of 1-formyl-3-(4-methoxyphenyl)cyclopentane **10**, hydrolyzes at a rate in between those of the diethyl acetals of phenylacetaldehyde and benzaldehyde. The 4-methoxyphenyl group has a minor electron-withdrawing effect: the diethyl acetal of formylcyclopentane **9** hydrolyzes only 1.75 times faster than does **10**.

Consistent with the electron-withdrawing stabilization effect is the observation that the substrate models containing poorly electron-withdrawing groups, such as **7–10**, hydrolyze to the corresponding aldehydes, but the inhibitor models which contain better electron-withdrawing groups, such as **11–13**, hydrolyze to the corresponding hydrates (by NMR). This again exemplifies the electron-withdrawing stabilization effect on the sp^3 hybridization of the hydrate carbon atom.

The magnitude of the stabilizing effect of the heterocyclic rings on acetal hydrolysis is quite remarkable. The difference in the greater stabilization effects of the heterocycles toward acetal hydrolysis relative to dialysis of the enzyme adducts apparently results from the different heteroatoms involved and the different conditions of the experiments. Nonetheless, the correlation of the stability of the enzyme adduct with this model can be used to the advantage of the medicinal chemist in the design of new classes of MAO inhibitors with varying degrees of reversibility. This effect also should be of interest to synthetic chemists. Acetals are common protecting groups for aldehydes because they are generally easily removed under mildly acidic conditions.[18] However, the results of these studies indicate that acetal protection of aldehydes adjacent to heteroatoms may lead to inordinately stable acetals, which may require very harsh conditions for deprotection.

Conclusions

The results described here support the hypothesis that the mechanism of inhibition of MAO by various heterocyclic compounds involves the formation of a covalent bond to an active site residue at the α-carbon of the inhibitor. This enzyme complex, which may resemble the structure of enzyme complexes with substrates, is stabilized relative to those with substrates by virtue of the electron-withdrawing ability of the heterocyclic rings. The magnitude of this effect is demonstrated by a corresponding stabilizing effect of these heterocycles on acetals.

Experimental Section

Analytical Methods and Reagents. NMR spectra were recorded either on a Varian EM-390 90-MHz, a Varian Gemini-300 300-MHz, or a Varian XLA-400 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$, unless stated otherwise. Thin-layer chromatography was performed on EM/UV silica gel plates with a UV indicator. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Elemental combustion analyses were performed by Oneida Research Services, Inc., Whitesboro, NY. Mass spectra were recorded on a VG Instruments VG70-250SE high-resolution spectrometer. Column chromatography was performed with Merck silica gel (230–400 mesh). All chemicals were purchased from Aldrich Chemical Co. and were used without further purification. Dichloromethane was dried by passing it through an alumina column. THF and ether were freshly distilled from sodium metal. Glassware was dried in an oven overnight when dry conditions were required. All reactions were carried out in an atmosphere of inert gas (nitrogen or argon).

Benzaldehyde Diethyl Acetal (7). A solution of benzaldehyde (530 mg, 5.0 mmol) and triethyl orthoformate (2.10 g, 14.2 mmol) in 10 mL of ethanol in the presence of a catalytic amount of p-toluenesulfonic acid was stirred at room temperature for 18 h. The reaction was quenched by the addition of 1.0 mL of saturated $NaHCO_3$, and then the mixture was concentrated in vacuo. The residue was redissolved in benzene, and the benzene solution was dried over $MgSO_4$. Removal of benzene and bulb-to-bulb distillation of the residue at 80–82 °C/7 mmHg (lit.[19] bp 75 °C/4 mmHg) afforded a colorless liquid (550 mg, 61%): ^{1}H NMR (300 MHz) δ 7.30–7.55 (m, 5 H), 5.58 (s, 1 H), 3.55–3.70 (m, 4 H), 1.30 (m, 6 H).

Phenylacetaldehyde Diethyl Acetal (8). The same procedure employed for the preparation of benzaldehyde diethyl acetal (**7**) was followed. The acetal **8** was obtained by bulb-to-bulb distillation at 85–88 °C/7 mmHg (lit.[10] bp 91–92 °C/8 mmHg) to give a colorless liquid (670 mg, 70%): ^{1}H NMR (300 MHz) δ 7.23 (m, 5 H), 4.62 (t, 1 H), 3.60 (m, 2 H), 3.40 (m, 2 H), 2.82 (d, 2 H), 1.10 (m, 6 H).

(Diethoxymethyl)cyclopentane (9). To a solution of t-BuOK (1.3 g, 11.6 mmol) in 25 mL of anhydrous THF at −50 °C in a dry ice/acetone bath was added (methoxymethyl)triphenylphosphonium chloride (4.1 g, 11.9 mmol). The cold bath was removed, and the temperature was allowed to rise slowly to room temperature. The mixture was stirred for 30 min at room temperature, it was then cooled to −50 °C, and a solution of 2-cyclopenten-1-one (0.7 g, 8.33 mmol) in 10 mL of anhydrous THF was added via syringe. The temperature was allowed to rise slowly to room

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temperature over 2 h, and then the mixture was stirred at room temperature overnight and poured into 30 mL of water. The aqueous solution was concentrated in vacuo, and the concentrated solution was extracted with cyclohexane (2 × 30 mL). The organic extracts were combined and dried over magnesium sulfate. Following removal of the solvent, the residue was distilled (110 °C/35 mmHg) to give a colorless liquid which was dissolved in 10 mL of absolute ethanol. To this solution was added 1.0 mL of triethyl orthoformate followed by the addition of a catalytic amount of *p*-toluenesulfonic acid. The mixture was stirred at room temperature overnight, and then the reaction was quenched with 1.0 mL of saturated sodium bicarbonate, concentrated in vacuo, and extracted with ether. The ether extracts were dried over magnesium sulfate. Following removal of the ether, the residue was bulb-to-bulb distilled at 85–87 °C/30 mmHg (lit.[20] bp 79–80.5 °C/17 mmHg) to give a colorless liquid (350 mg, 25%): ^{1}H NMR (400 MHz) δ 4.22 (d, 1 H), 3.65 (m, 2 H), 3.55 (m, 2 H), 2.20 (m, 1 H), 1.30–1.80 (m, 8 H), 1.25 (m, 6 H); ^{13}C NMR (100 MHz) δ 106.95, 61.30, 42.87, 28.77, 25.98, 15.77.

3-(4-Methoxyphenyl)cyclopentanone (15). A solution of 4-bromoanisole (3.8 g, 20 mmol) in 50 mL of anhydrous ether was treated with *n*-butyllithium (2.0 M, 10 mL, 20 mmol) at 0 °C under argon. The solution was stirred at 0 °C for 30 min before it was transferred to another round-bottomed flask containing a suspension of Cu_2Br_2 in 25 mL of anhydrous ether at 0 °C. Upon completion of the transfer, 2-cyclopenten-1-one (0.83 mL, 10 mmol) was added dropwise via syringe. The mixture was kept at 0 °C with stirring for 1 h, and then 25 mL of 1 N HCl was introduced to quench the reaction. The ether layer was separated, the aqueous layer was extracted with ether (2 × 30 mL), and the combined extracts were washed with saturated $NaHCO_3$. After it was dried over $MgSO_4$, the ether was removed in vacuo. The residue was purified by silica gel column chromatography to give 800 mg (42%) of colorless crystals: mp 47–49 °C (lit.[11] mp 46–48 °C); ^{1}H NMR (400 MHz) δ 7.17 (d, J = 9 Hz, 2 H), 6.90 (d, J = 9 Hz, 2 H), 3.80 (s, 3 H), 3.40 (m, 1 H), 2.65 (dd, J_1 = 8 Hz, J_2 = 19 Hz, 1 H), 2.25–2.51 (m, 4 H), 1.95 (m, 1 H); FTIR (KBr) 1740 cm^{-1}; HRMS calcd for $C_{12}H_{14}O_2$ 190.0994, found 190.0980.

1-Formyl-3-(4-methoxyphenyl)cyclopentane Diethyl Acetal (10). To a solution of potassium *tert*-butoxide (530 mg, 4.72 mmol) in 25 mL of anhydrous THF at −10 °C under argon was added (methoxymethyl)-triphenylphosphonium chloride (1.63 g, 4.75 mmol). The mixture was stirred at −10 °C for 20 min, and then a solution of 3-(4-methoxyphenyl)-cyclopentanone (300 mg, 1.58 mmol) in anhydrous THF was introduced dropwise via syringe. The resulting mixture was stirred at −10 °C for 2 h and at room temperature for 3 h, and then it was poured into 50 mL of water. The aqueous solution was extracted with ethyl acetate (2 × 30 mL), and the combined extracts were washed with water (2 × 50 mL) and brine (50 mL) and dried over $MgSO_4$. Following removal of the solvent, the residue was chromatographed on a silica gel column (cyclohexane/ethyl acetate, 4:1) to give a light oil, which was dissolved in 10 mL of ethanol. To this solution was added triethyl orthoformate (600 mg, 4.0 mmol) followed by a catalytic amount of *p*-toluenesulfonic acid (5.0 mg). The resulting mixture was stirred at room temperature for 18 h, and then the reaction was quenched by the addition of 2 mL of saturated $NaHCO_3$. The solvents were concentrated in vacuo, the residue was resuspended in ethyl acetate, and the solution was washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL). After it was dried over $MgSO_4$, the ethyl acetate solution was concentrated in vacuo. The resulting solution was eluted through a column of silica gel (4:1 hexane/ethyl acetate), which was neutralized by preparation in 4:1 hexane/ethyl acetate containing 1% triethylamine. The combined fractions of the desired product were concentrated, and the product was further purified by bulb-to-bulb distillation at 165–170 °C/5 mmHg to give a colorless liquid as a mixture of two diastereoisomers (320 mg, 73%): ^{1}H NMR (400 MHz) δ 7.20 (t, 2 H), 6.88 (d, 2 H), 4.37 (d, 1 H), 3.85 (s, 3 H), 3.70 (m, 2 H), 3.60 (m, 2 H), 3.05 (m, 1 H), 2.50 (m, 1 H), 1.40–2.20 (m, 6 H), 1.25 (m, 6 H); FTIR (neat) 2972.5 (s), 2872 (s), 1612.6 (m), 1512.3 (s), 1462.1 (m), 1248 (s), 1178.6 (m), 1120.7 (s), 1060 (s) cm^{-1}; HRMS calcd for $C_{17}H_{26}O_3$ 278.1882, found 278.1848; MS (EI) m/z 278 (M^+, 11.4), 233 (24), 232 (100), 203 (9.3), 187 (42), 186 (50), 173 (59), 134 (44.2), 121 (42.3), 103 (91), 98 (33), 91 (16), 85 (17), 75 (35), 70 (20.5), 47 (25), 40 (26).

1-(4-Methoxyphenyl)-5-oxopyrrolidine-3-carboxylic Acid (19). A solution of itaconic acid (**18**, 24.8 g, 0.191 mol) and anisidine (23.5 g, 0.191 mol) in 190 mL of deionized water was heated at reflux for 3 h. The resultant solution was slowly cooled to room temperature, and pinkish crystals (43.2 g, 97%) were collected and washed with cold deionized water: mp 168–170 °C; ^{1}H NMR (90 MHz, $CDCl_3$/DMSO) δ 7.45 (d, 2 H), 6.87 (d, 2 H), 6.05 (b.s), 4.00 (d, 2 H), 3.78 (s, 3 H), 3.32 (m, 1 H), 2.80 (dd, 2 H); IR (KBr) 3020 (broad, s), 1725 (s), 1646 (s) cm^{-1}; HRMS (EI) calcd for $C_{12}H_{13}NO_4$ 235.0844, found 235.0840.

Methyl 1-(4-Methoxyphenyl)-5-oxopyrrolidine-3-carboxylate (20). To a solution of **19** (21 g, 89.3 mmol) in 100 mL of methanol under nitrogen at 0 °C was added thionyl chloride (7.8 mL, 107 mmol) via syringe. The mixture was stirred at 0 °C for 1.5 h, and then the temperature was allowed to rise to room temperature. Stirring continued at room temperature for 3 h, and then the solvent was removed in vacuo and the solid residue was distilled at 220–230 °C/2 mmHg. The product was obtained as a crystalline solid (20.7 g, 93%): mp 86–88 °C; ^{1}H NMR (90 MHz) δ 7.46 (d, 2 H), 6.90 (d, 2 H), 4.03 (dd, 2 H), 3.75 (s, 3 H), 3.73 (s, 3 H), 3.35 (m, 1 H), 2.85 (dd, 2 H); IR (KBr) 1733 (s), 1691 (s), 1513 (s) cm^{-1}; HRMS (EI) calcd for $C_{13}H_{15}NO_4$ 249.1001, found 249.1001. Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.65; H, 6.02; N, 5.62. Found: C, 62.26; H, 6.04; N, 6.22.

4-(Hydroxymethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone (21). To a solution of **20** (1.1 g, 4.4 mmol) in 40 mL of methanol under nitrogen at 0 °C was added sodium borohydride (335 mg, 8.8 mmol) portionwise. The solution was heated at reflux for 3 h, the resulting mixture was then cooled to 0 °C, and the residual sodium borohydride was destroyed by successive additions of deionized water (5 mL) and 10% HCl (5 mL). Following concentration in vacuo, the residue was extracted with ethyl acetate (3 × 30 mL), and the combined extracts were washed with saturated sodium bicarbonate solution and dried over $MgSO_4$. The solvent was removed, and the crude product was purified by silica gel column chromatography (15 g of silica gel, ethyl acetate, R_f = 0.25), affording **21** as colorless crystals (0.84 g, 86%): mp 111–113 °C; ^{1}H NMR (400 MHz) δ 7.50 (d, 2 H), 6.90 (d, 2 H), 3.92 (dd, 1 H), 3.80 (s, 3 H), 3.71 (m, 2 H), 2.70 (m, 2 H), 2.41 (d, 1 H), 1.89 (m, 1 H), 1.64 (s, 1 H); IR (KBr) 3361 (broad, s), 1647 (s), 1515 (s) cm^{-1}; HRMS (EI) calcd for $C_{12}H_{15}NO_3$ 221.1052, found 221.1051. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.16; H, 6.79; N, 6.33. Found: C, 65.14; H, 6.80; N, 6.25.

4-Formyl-1-(4-methoxyphenyl)-2-pyrrolidinone (22). To a solution of **21** (200 mg, 0.9 mmol) dissolved in 30 mL of anhydrous CH_2Cl_2 under argon was added pyridinium chlorochromate (190 mg, 0.88 mmol). The mixture was stirred for 1 h at room temperature, and then the solution was diluted with hexane and Celite was added. The suspension was stirred at room temperature for 2 h before it was filtered through a bed of Celite. The filtrate was concentrated in vacuo, and the residue was passed through a short silica gel column (ethyl acetate as eluant) to give a light yellowish oil (150 mg, 76% yield): ^{1}H NMR (400 MHz), δ 9.80 (s, 1 H), 7.47 (d, J = 15 Hz, 2 H), 6.91 (d, J = 15 Hz, 2 H), 4.19 (dd, J_1 = 4.6 Hz, J_2 = 10 Hz, 1 H), 3.99 (dd, J_1 = 8.5 Hz, J_2 = 10 Hz, 1 H), 3.81 (s, 3 H), 3.33 (m, 1 H), 2.91 (dd, J_1 = 7.1 Hz, J_2 = 9 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.19, 171.18, 157.49, 132.07, 122.68, 114.59, 55.87, 48.16, 42.84, 32.55; HRMS calcd for $C_{12}H_{13}NO_3$ 219.0895, found 219.0896. Anal. Calcd for $C_{18}H_{18}N_5O_6$ (2,4-dinitrophenylhydrazone): C, 54.00; H, 4.50; N, 17.50. Found: C, 53.83; H, 4.25; N, 17.37.

4-(Diethoxymethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone (11). Triethyl orthoformate (300 mg, 2.0 mmol) was added to a solution of **22** (220 mg, 1.0 mmol) in 1.0 mL of anhydrous ethanol, followed by the addition of *p*-toluenesulfonic acid (1.0 mg). The mixture was stirred at room temperature for 2 h, at which time TLC analysis indicated that the starting material had been consumed (R_f = 0.2, ethyl acetate) and a new, less polar spot appeared (R_f = 0.55). The reaction was quenched by addition of saturated sodium bicarbonate solution (0.5 mL) followed by 25 mL of benzene. Celite was added to the mixture, and the suspension was stirred for 1 h before it was filtered through a bed of Celite. The filtrate was concentrated in vacuo, and the residue was purified by deactivated (Et_3N) silica gel column chromatography (ethyl acetate/hexane, 2:1) to give a colorless oil (190 mg, 65% yield): ^{1}H NMR (300 MHz) δ 7.50 (d, J = 10 Hz, 2 H), 6.90 (d, J = 10 Hz, 2 H), 4.48 (d, J = 6.6 Hz, 1 H), 3.80 (s, 3 H), 3.50–3.90 (m, 7 H), 2.70 (m, 2 H), 1.60 (m, 1 H), 1.20 (m, 6 H); HRMS calcd for $C_{16}H_{23}NO_4$ 293.1627, found 293.1610. Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.53; H, 7.85; N, 4.78. Found: C, 65.28; H, 7.91; N, 4.68.

Glycidaldehyde Diethyl Acetal (23). To a stirred solution of acrolein diethyl acetal (26 g, 0.2 mol) in 100 mL of methanol was added sodium bicarbonate (3.0 g, 35.7 mmol) followed by benzonitrile (21 mL, 0.204 mol) and 30% hydrogen peroxide (23 mL, 0.203 mol) at room temperature. The mixture was stirred overnight, and then it was heated at 50 °C for 5 h. Water (50 mL) was added, and stirring was maintained for 3 h. The solution was concentrated in vacuo, and the residue was extracted with

(20) De Botton, M. *Bull. Soc. Chim. Fr.* **1976**, 849–856.

CH_2Cl_2 (2 × 100 mL). The combined extracts were dried over magnesium sulfate, the CH_2Cl_2 was evaporated, and the residue was diluted with hexane. After it was filtered through a bed of Celite, the resulting filtrate was concentrated in vacuo, and the crude product was distilled in vacuo at 110 °C/25 mmHg to give a colorless liquid (14 g, 48%).

5-(Diethoxymethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (12). To a solution of *p*-methoxyphenylacetic acid (**24**, 1.7 g, 10.2 mmol) in anhydrous THF was added a 2.4 M solution of *n*-BuLi (8.5 mL, 20.4 mmol) at 0 °C under argon. The resulting solution was allowed to warm to room temperature, and stirring was maintained for 30 min before HMPA (1.8 mL) was added. To the yellowish solution was added glycidaldehyde diethyl acetal (**23**, 1.5 g, 10.3 mmol) at room temperature. The mixture was stirred for 3 h at room temperature and at reflux for 3 h, and then after the solution was cooled to room temperature, water and saturated ammonium chloride (10 mL each) were added and the solution was stirred overnight. Following concentration in vacuo the residue was extracted with ethyl acetate, and the combined organic extracts were extracted with a 10% solution of sodium hydroxide (20 mL). The aqueous basic extract was washed with chloroform and was acidified first with acetic acid then with 10% aqueous HCl to pH 2.5. The acidified solution was extracted with ethyl acetate, the organic extracts were dried over magnesium sulfate, and the solvent was removed in vacuo to afford a colorless oil. The oil was dissolved in benzene (50 mL), a catalytic amount of *p*-toluenesulfonic acid was added, and the resulting solution was heated under reflux for 3 h to remove water azeotropically with a Dean–Stark trap. The solution was cooled, extracted with saturated $NaHCO_3$ (30 mL), and dried over $MgSO_4$. Removal of the benzene gave a colorless oil, which was purified by silica gel column chromatography to give a mixture of two diastereomers as a colorless oil (1.2 g, 40%): ^{1}H NMR (400 MHz) δ 7.25 (2 sets of doublets, 2 H), 6.92 (2 sets of doublets, 2 H), 4.67 (2 sets of doublets, 1 H), 4.52–4.64 (m, 1 H), 3.80–4.10 (m, 3 H), 3.80 (s, 3 H), 3.65 (m, 2 H), 2.30–2.90 (m, 2 H), 1.30 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.57, 177.37, 159.39, 159.26, 144.69, 144.60, 130.44, 129.61, 129.35, 128.74, 103.27, 102.76, 77.85, 77.64, 66.44, 65.22, 64.76, 64.17, 55.68, 45.66, 45.19, 31.96, 31.70, 15.93, 15.81. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.31; H, 7.48. Found: C, 65.23; H, 7.59.

2-Hydroxy-3-[(4-methoxyphenyl)amino]propanal Diethyl Acetal (27). A mixture of anisidine (**26**, 1.01 g, 8.2 mmol) and glycidaldehyde diethyl acetal (**23**, 0.6 g, 4.1 mmol) was heated in an oil bath at 60 °C for 5 h. The mixture was cooled to room temperature, and the residue was distilled at 160 °C/1 mmHg to give a yellowish oil (0.6 g, 54%): ^{1}H NMR (400 MHz) δ 6.77 (d, 2 H), 6.62 (d, 2 H), 4.47 (d, 1 H), 3.80 (m, 2 H), 3.75 (s, 2 H), 3.60 (m, 2 H), 3.32 (m, 1 H), 3.15 (m, 1 H), 1.25 (m, 6 H); ^{13}C NMR (100 MHz) δ 152.73, 142.97, 115.23, 115.11, 104.16, 70.80, 64.47, 64.13, 56.20, 53.40, 46.53, 15.85; FTIR (neat) 3380 (broad, s) cm^{-1}; HRMS calcd for $C_{14}H_{23}NO_4$ 269.1627, found 269.1610.

5-(Diethoxymethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (13). To a solution of **27** (500 mg, 1.86 mmol) in 50 mL of anhydrous THF was added carbonyldiimidazole (600 mg, 3.72 mmol). The mixture was heated at reflux for 5 h (TLC indicated that the starting material was completely consumed: R_f = 0.40, ethyl acetate/hexane, 2:1). The reaction mixture was cooled to room temperature, 0.5 mL of saturated $NaHCO_3$ was added, and the resulting suspension was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (2:1 ethyl acetate/hexane, R_f = 0.43), giving **13** as a light yellow oil (510 mg, 93%): ^{1}H NMR (400 MHz) δ 7.45 (d, J = 9 Hz, 2 H), 6.90 (d, J = 9 Hz, 2 H), 4.65 (d, J = 4 Hz, 1 H), 4.59 (m, 1 H), 3.98 (m, 2 H), 3.82 (m, 2 H), 3.80 (s, 3 H), 3.65 (m, 2 H), 1.23 (m, 6 H); ^{13}C NMR (400 MHz) δ 156.69, 155.23, 131.84, 120.64, 114.63, 102.20, 72.64, 65.67, 64.40, 55.90, 46.40, 15.82, 15.64; FTIR (neat) 1754 cm^{-1}; HRMS calcd for $C_{15}H_{21}NO_5$ 295.1420, found 295.1421. Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.02; H, 7.12; N, 4.75. Found: C, 60.89; H, 7.16; N, 4.65.

Determination of the Rates of Acetal Hydrolysis. A typical experiment was as follows: The acetal (20 mg) was dissolved in a 2:1 mixture of acetone-d_6 and a 0.5% DCl solution in D_2O (0.5 mL) in a NMR tube at 23 °C. The progress of the hydrolysis was monitored by integration of the formation of the aldehyde protons (or the hydrate protons in the case of **11–13**) and the decrease in the ethyl acetal protons.

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