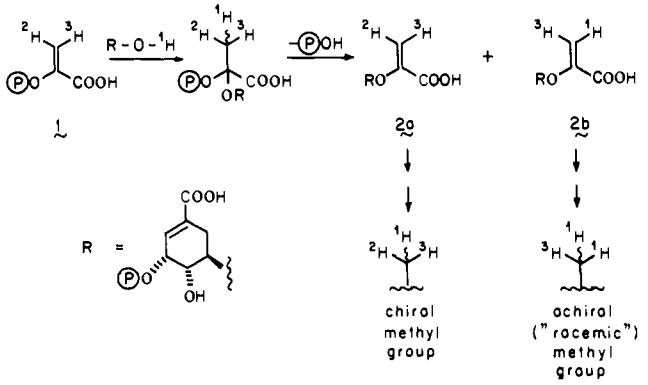
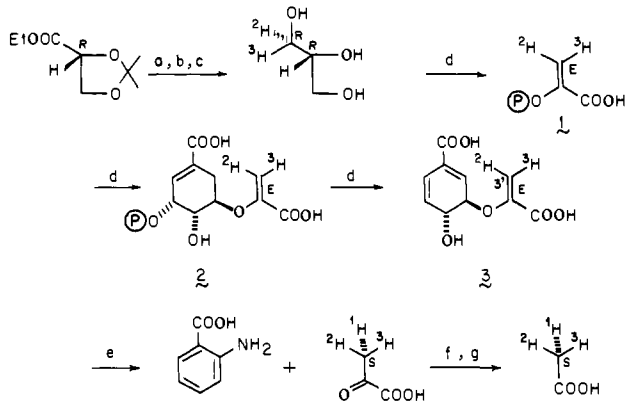
Scheme I



Scheme II[a]



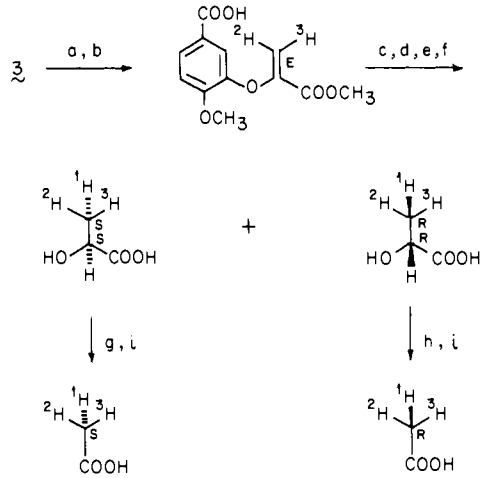
[a] (a) $LiAlD_4$; (b) liver alcohol dehydrogenase, [1-^{3}H]ethanol; (c) H^+; (d) *Klebsiella pneumoniae* 62-1; (e) anthranilate synthetase; (f) lactate dehydrogenase, NADH; (g) $Cr_2O_7^{2-}$, H^+.

same stereochemistry, e.g., both anti, or *E* configuration if the two steps proceed with opposite stereochemistry, i.e., one syn and one anti. To determine if it was indeed chirally labeled, aliquots of the product **3** were subjected to the action of anthranilate synthetase[13] in the presence of excess lactate dehydrogenase and NADH. The resulting lactate was oxidized to acetate,[14] which was analyzed for the configuration of the methyl group by the method of Cornforth et al.[15] and Arigoni and co-workers.[16] Observed *F* values[17] of 44.0 and 45.1 indicated the presence of 19% enantiomeric excess (ee) of *S* methyl groups.

The double-bond configuration of the deuterated, tritiated **3** was then determined by the reaction sequence shown in Scheme III. Aromatization,[18] reduction with Wilkinson's catalyst,[19] and Birch reduction[20] followed by hydrolysis gave a racemic mixture of lactate (10% yield) in which by virtue of the cis addition of hydrogen the configurations at C-2 and C-3 are correlated with each other. Incubation of an aliquot of the lactate with L-lactate dehydrogenase/NAD^+, followed by H_2O_2 oxidation of the resulting pyruvate[21] gave acetate, which in two separate experiments showed *F* values of 45.5 and 44.5 (16% and 19% ee *S*). Conversely, aliquots of the lactate upon oxidation with D-lactate dehydrogenase/NAD^+ and H_2O_2 gave acetate of $F = 55.1$ and 56.4 (18% and 22% ee *R*).

The results establish predominant *E* configuration for the side-chain double bond of the deuterated, tritiated **3** generated

Scheme III[a]



[a] (a) Pyridinium dichromate; DMF; (b) CH_2N_2; (c) tris(triphenylphosphine)rhodium chloride, H_2, benzene; (d) NaOH, CH_3OH; (e) Na, liquid NH_3; (f) dilute HCl, reflux; (g) L-lactate dehydrogenase, NAD^+; (h) D-lactate dehydrogenase, NAD^+; (i) H_2O_2.

from (*E*)-[3-$^2H_1$,$^3H_1$]-**1**, indicating that the addition and elimination steps in the ESP synthetase reaction proceed with opposite stereochemistry. This finding is consistent with a reaction path requiring a minimum of motion during the catalytic process. With the caveat that only one enantiomeric data set is available, the results also suggest that anthranilate synthetase catalyzed protonation on the *re* face of the enolpyruvyl side chain of **3**. This contrasts with the steric course of the reactions studied which involve attack at C-3 of phosphoenolpyruvate.[22]

Acknowledgment. This work was supported by USPHS Research Grants GM 18852 and GM 32333.

(13) Egan, A. F.; Gibson, F. *Methods Enzymol.* **1970**, *17A*, 380.
(14) Simon, H.; Floss, H. G. "Bestimmung der Isotopenverteilung in markierten Verbindungen"; Springer: Berlin, 1967; p 50.
(15) Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutschow, C. *Eur. J. Biochem.* **1970**, *14*, 1.
(16) Lüthy, J.; Rêtey,J.; Arigoni, D. *Nature (London)* **1969**, *221*, 1213.
(17) Floss, H. G.; Tsai, M.-D. *Adv. Enzymol.* **1979**, *50*, 243.
(18) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(19) Fryzuk, M. D.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6262.
(20) Birch, A. J.; Sternhell, S. *Aust. J. Chem.* **1954**, *7*, 256.
(21) Rose, I. A. *J. Biol. Chem.* **1970**, *245*, 6052.
(22) Rose, I. A. *CRC Crit. Rev. Biochem.*, **1972**, *1*, 33.

Copper-Catalyzed Acylation and Conjugate Addition of Zinc Homoenolate. Synthesis of 4- and 6-Oxo Esters

Eiichi Nakamura* and Isao Kuwajima*

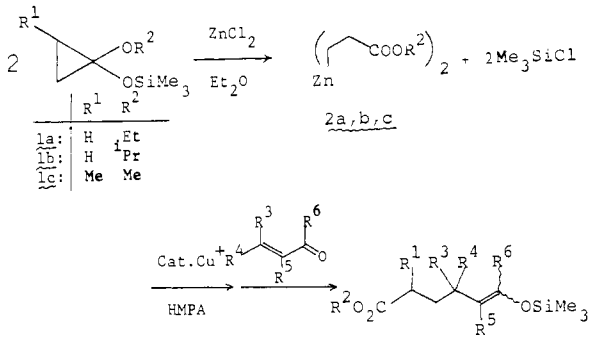
Department of Chemistry
Tokyo Institute of Technology
Meguro, Tokyo 152, Japan
Received February 8, 1984

Formation of carbon–carbon bonds by way of homoenolate anion has been a long-standing desire for organic chemists. A large portion of such an endeavor, some with the anion itself and usually with its equivalents, has been concentrated on 1,2-addition onto carbonyl compounds.[1] Feasibility and potentiality of conjugate addition, however, have not yet been demonstrated.[2] We are pleased to record here the first successful realization of such a reaction made possible by a copper-catalyzed reaction of zinc homoenolate **2** (eq 1). An expeditious synthetic route to γ-keto esters by C-acylation of the copper species is also reported.

The method for the conjugate addition is very simple yet highly efficient, consisting of in situ preparation of a mixture of the zinc

(1) For instance: (a) Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1977**, *99*, 7360; *Ibid.* **1983**, *105*, 651. (b) Goswami, R.; Corcoran, D. E. *Tetrahedron Lett.* **1982**, *23*, 1463. (c) Caine, D.; Frobese, A. S. *Ibid.* **1978**, 883. (d) Review: Werstiuk, N. H. *Tetrahedron* **1983**, *39*, 205.
(2) (a) With "equivalents"; Lesur, B.; Toye, J.; Chantrenne, M.; Ghosez, L. *Tetrahedron Lett.* **1979**, 2835. De Lombaert, S.; Lesur, B.; Ghosez, L. *Ibid.* **1982**, *23*, 4251. Evans, D. A.; Billargeon, D. J.; Nelson, T. V. *J. Am. Chem. Soc.* **1978**, *100*, 2242. (b) Homoenolate "radicals" add to enones of limited structural types: Giese, B.; Horner, H. *Tetrahedron Lett.* **1983**, *24*, 3221.

0002-7863/84/1506-3368$01.50/0



homoenolate and chlorotrimethylsilane (Me_3SiCl) from the cyclopropane **1**[1a] and $ZnCl_2$[3] followed by successive addition of a catalytic amount (5 mol %) of $CuBr{\cdot}Me_2S$ complex, hexamethylphosphorotriamide (HMPA), and an unsaturated carbonyl compound at 0 °C-room temperature. Nonaqueous workup gives the desired 6-oxo ester as its enol silyl ether with the double-bond specifically (>>99%) at the mechanistically expected position (eg 1; see Scheme I as a proof of the regiochemistry). The representative results are summarized in Table I. The reaction applies equally well to enones (entries 1–5), enals (entry 6), and some acetylenic carbonyl compounds (entries 7, 8). All reactions examined were very clean; the crude mixture consisted of the adduct and small amounts (< ca. 10%) of the starting **1** and a dimerization product, e.g., diethyl adipate. The reaction of the methylcyclopropane **1c** gave only the α-methyl ester adduct (entry 3).[4] The reaction proceeded exclusively via the 1,4-addition pathway (cf. entry 4) except the case with an enal (entry 6) where a small portion (<3%) of the adduct arised from 1,2-addition. No evidence of O-alkylation reaction[3b] could be found. The reaction of an ynone cleanly gave a hydrolytically unstable siloxyallene (entry 7), which serves as a useful precursor to substituted enones. Hydrolysis gave a 1:1 stereoisomeric mixture of the parent enone. The reaction of dimethyl acetylenedicarboxylate mainly gave a single isomer (presumably *Z*.

The following procedure is typical: A mixture of $ZnCl_2$ (1.022 g, 7.5 mmol) and **1a** (1.74 g, 10.0 mmol) in 15 mL of ether was stirred for 3 h (under ultrasonic irradiation, if necessary).[3a] $CuBr{\cdot}Me_2S$ (31 mg, 0.15 mmol), 2-cyclohexen-1-one (481 mg, 5.0 mmol), and HMPA (1.74 mL, 10.0 mmol) was added sequentially at 0 °C. After 4 h at 20 °C, 2 g of silica gel was added. The mixture was diluted with hexane and filtered through a pad of silica gel. The 1,4-adduct (1.03 g, 76%) was obtained as its enol silyl ether by distillation.

Synthetic value of the silylated 1,4-adducts is evident; the silyl group can be removed readily, if so desired, by wet ether (overnight stirring) or may be exploited for a number of subsequent synthetic operations.[5] Examples in Scheme I illustrate the latter possibilities in the synthesis of a (protected) hydroxy ketone and a keto lactam.

Findings pertaining to the reaction mechanism involve that all of the reagents present in the reaction mixture, including HMPA and Me_3SiCl, are the absolute necessity for the smooth conjugate addition. Inefficiency of ligands such as Bu_3P and Me_2S implies the primary role of HMPA to promote the initial transmetalation to copper. Of particular note is *the striking effect of Me_3SiCl, which is generated during the preparation of* **2**; *without this*

Scheme I

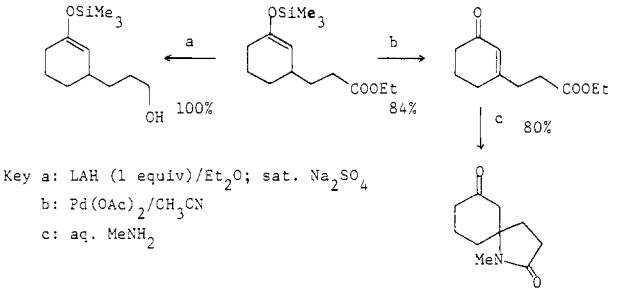
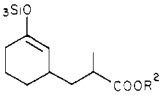
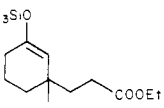
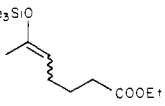
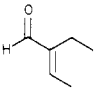
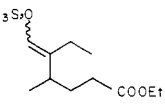
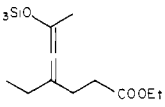
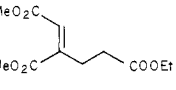


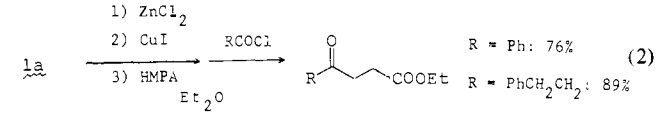
Table I. Conjugate Addition of Homoenolate of Esters[a]

entry	acceptor	product	% yield[b]
1, R^1 = H; R^2 = *i*-Pr			93 (100)[c]
2, R^1 = H; R^2 = Et			76
3, R^1 = Me; R^2 = Me			91
4			92
5			80[d]
6			75[e]
7			73
8			63[f]

[a] The reaction (0.75–5-mmol scale) were performed with 1 equiv of **2a** except entry 1 (with **2b**), entry 3 (**2c**), and entry 4 (2 equiv of **2a**). [b] Isolated yield after column chromatography or distillation. [c] GLC yield. [d] ^1H NMR and GLC analyses indicated a *E*/*Z* ratio of 24:76. [e] The ratio of stereoisomers (unassigned) was ca. 91:9. [f] The product appeared to remain unsilylated in the reaction mixture.

occurred almost no reaction even in the presence of HMPA. Me_3SiCl is suspected to activate the enone toward the copper species.[6,7] Studies on the stoichiometry of the reactants suggested that only one of the two homoenolate moieties in **2** can be transmetalated to copper.

The copper homoenolate species prepared under similar conditions reacts rapidly with acid chlorides via an exclusive C-acylation pathway[3b] (eq 2). The reaction provides one of the



simplest preparative routes to the synthetically important γ-keto esters.[8]

(3) (a) Pure **2** can be obtained in the following manner. The crude ethereal solution of **2** and Me_3SiCl is concentrated in vacuo to leave a semisolid residue, which was diluted with a small amount of methylene chloride, then with a large amount of hexane. Supernatant is taken and concentrated to leave **2** as an oil: a procedure developed by J. Shimada, who also performed characterization. ^1H NMR ($CDCl_3$) 0.29 (t, $J \simeq 7.5$ Hz, 2 H), 1.27 (t, $J = 7$, 3 H), 2.66 (t, $J = 7.5$ Hz, 2 H), 4.20 (g, $J = 7$ Hz, 2 H); ^{13}C NMR ($CDCl_3$) 2.7 (t), 14.2 (q), 32.4 (t), 61.7 (t), 184.2 (s); IR ($CDCl_3$, 0.06 M) 1647 (s), 1720 (w). (b) A striking behavior of **2** involves O-acylation forming an (acyloxy)cyclopropane upon reaction with an acid chloride under certain conditions: Oshino, H., unpublished results.

(4) The methylcyclopropane **1b** was prepared by reductive silylation (Na/Me_3SiCl) of methyl 3-chloro-2-methylpropanoate. The regiochemistry of the homoenolate formation will be discussed in due course.

(5) Brownbridge, P. *Synthesis* **1983**, *1*: *Ibid.* **1983**, *85*.

(6) Cf.: Yamamoto, Y.; Yamamoto, S.; Yatagai, H.; Ishihara, Y.; Maruyama, K. *J. Org. Chem.* **1982**, *47*, 119.

(7) The HMPA–Me_3SiCl system seems to have its own virtue in organocopper chemistry and this point is under investigation.

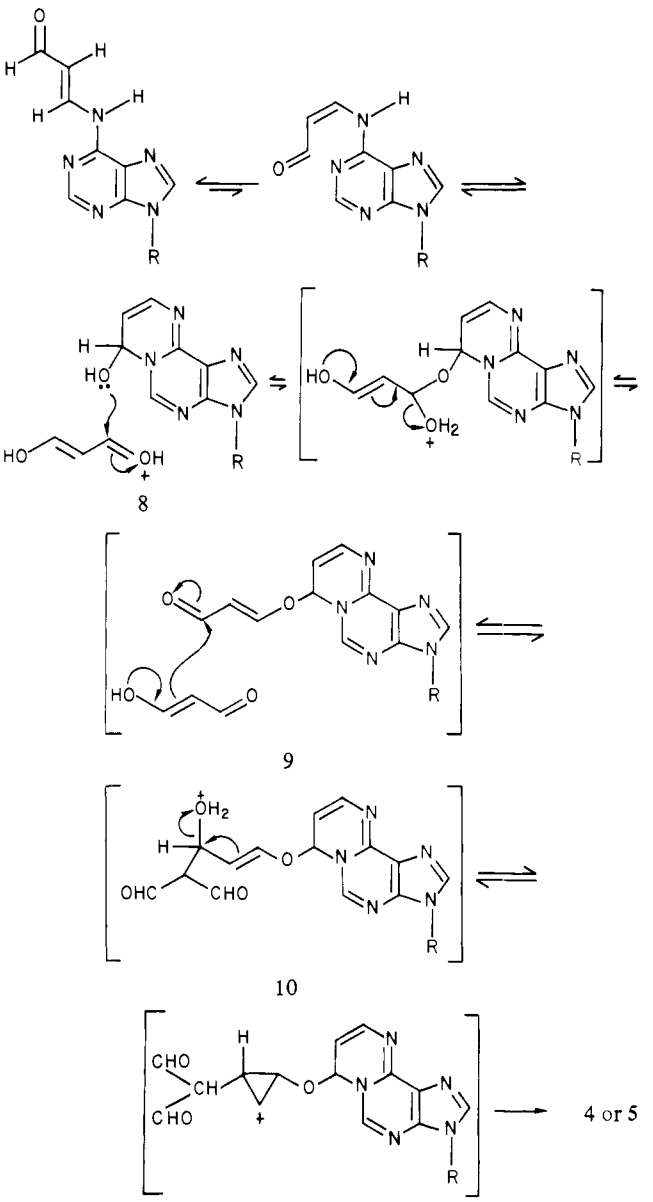
(8) Cf.: Shimada, J.; Hashimoto, K.; Kim, B.-Y.; Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1984**, *106*, 1759.

The copper-catalyzed conditions reported here work successfully with enones, enals, acetylenic carbonyl compounds, and acid chlorides, but aldehydes, alkyl acrylates, epoxides, and allyl acetates were found to be unreactive under such conditions. The 1,4-addition reaction could be performed in the presence of an aldehyde, no trace of ternary coupling products being detected. In the absence of Me_3SiCl, the conjugate addition is much slower than the acylation reaction; thus, a competitive experiment between benzoyl chloride and cyclohexenone gave only the γ-keto ester. The reactivity spectrum shown above is considerably different from the usual copper reagents.[7]

Even with the limited number of cases examined here, it seems already clear that the copper-catalyzed reactions of the zinc homoenolate will prove synthetically valuable.

(9) Cf.: Posner, G. "An Introduction to Synthesis Using Organocopper Reagents"; Wiley: New York, 1980.

Scheme I



Novel Adducts from the Modification of Nucleic Acid Bases by Malondialdehyde[1]

Vasu Nair,* Gregory A. Turner, and Rick J. Offerman

Department of Chemistry, University of Iowa
Iowa City, Iowa 52242

Received January 31, 1984

The ubiquitous natural compound, malondialdehyde (MDA) (**1**), is produced in animal tissues as an end product of unsaturated lipid peroxidation and as a side product of prostaglandin and thromboxane biosynthesis.[2] It is readily formed in the γ-irradiation of carbohydrates.[3] The reported toxicity and degenerative chemistry of MDA[4-8] may be a result of its ability to covalently bond and to cross-link a variety of biological macromolecules. Thus, the presence of MDA in foods and in living tissues in which the lipid component has undergone oxidation may be of considerable physiological importance. Malondialdehyde is reactive toward nucleic acids resulting in the loss of their template activity.[9,10] We have shown previously that MDA reacts relatively rapidly (as evidenced from kinetic data) at the α-amino group of amino acids to form both 1:1 and 1:2 adducts.[11] This communication reports on the isolation and structural elucidation of novel and unusual adducts from the reaction of MDA with adenine and cytosine.[12]

The reaction of MDA (as its sodium salt)[11] with adenosine was carried out in aqueous solution at pH 4.2[13] and 37 °C for 3 days to furnish two adducts, which were separated and purified by multiple reverse phase HPLC on Amberlite XAD-4 resin (40–50 μm) using ethanol/water as the eluting solvent. The first adduct, mp 125–127 °C, formed in about 7.0% conversion, showed UV absorption at $\lambda_{max}^{H_2O}$ 326 (ϵ = 46 000), 241 (ϵ = 8500), and 222 nm (ϵ = 9900). The presence of a molecular ion at m/z 321 and fragments in the mass spectrum and the UV data suggested the formation of a nucleoside modified at the 6-position by an α,β-unsaturated aldehyde moiety. The 360-MHz high-field ^1H NMR data (including homonuclear decoupling) together with the 90.6-MHz ^{13}C NMR data in Me_2SO-d_6 provided excellent supporting evidence for the complete structure and stereochemistry as **2**. The NH resonance appeared at δ 11.36 (d, J = 11.4 Hz) and the aldehyde proton at δ 9.42 (d, J = 8.5 Hz). The two vinyl protons gave resonances at δ 6.01 (d, d, $J_{c,d}$ = 8.5, $J_{b,c}$ = 13.3 Hz, H_c) and 8.71 (d, d, $J_{b,c}$ = 13.3, $J_{a,b}$ = 11.4 Hz, H_b) indicative of a trans geometry. The adenine ring protons appeared as singlets at δ 8.71 (H_2) and 8.56 (H_8). The ribose protons gave the expected resonance pattern with the anomeric proton appearing as a doublet at δ 6.01 (J = 5.7 Hz). The ^{13}C NMR spectrum of **2** showed 13 carbons with appropriate chemical shifts. The spectral data also suggest that the enaminal moiety in **2** is coplanar with the purine ring, and the marked downfield shift of the N–H is due largely to the diamagnetic anisotropic deshielding by the purine ring. An adduct similar to **2** was isolated (20%) as the single product from the reaction of methylmalondialdehyde (MMDA) and adenosine.

The second adduct (mp 149–151 °C, 11%) exhibited UV absorbances in H_2O at 327 (ϵ = 29 700), 260 (sh, ϵ = 13 960), and

(1) Presented in part at the 186th National Meeting of the American Chemical Society, Washington, DC, Aug 29, 1983.
(2) Pryor, W. A. "Free Radicals in Biology"; Academic Press: New York, 1976; Vol. I and II.
(3) Bucknall, T.; Edwards, H. E.; Kemsley, K. G.; Moore, J. S.; Phillips, G. O. *Carbohydr. Res.* **1978**, *62*, 49.
(4) Mukai, F. H.; Goldstein, D. B. *Science (Washington, D.C.)* **1976**, *191*, 868.
(5) Munkres, K. P. *Mech. Ageing Dev.* **1979**, *10*, 249.
(6) Chio, K. S.; Tappel, A. L. *Biochemistry* **1969**, *8*, 2821, 2827.
(7) Marnett, L. J.; Tuttle, M. A. *Cancer Res.* **1980**, *40*, 276.
(8) Hartman, P. E. *Environ. Mutagen.* **1983**, *5*, 603.
(9) Brooks, B. R.; Klamerth, O. L. *Eur. J. Biochem.* **1968**, *5*, 178.
(10) Summerfield, F. W.; Tappel, A. L. *Anal. Biochem.* **1981**, *111*, 77.
(11) Nair, V.; Vietti, D. E.; Cooper, C. S. *J. Am. Chem. Soc.* **1981**, *103*, 3030.
(12) Cf.: Moschel, R. C.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 294.
(13) Reaction occurs at a pH range of 4.0–7.0. However, the optimum conditions for reaction are at pH 4.2, slightly below the pK_a of MDA.

0002-7863/84/1506-3370$01.50/0