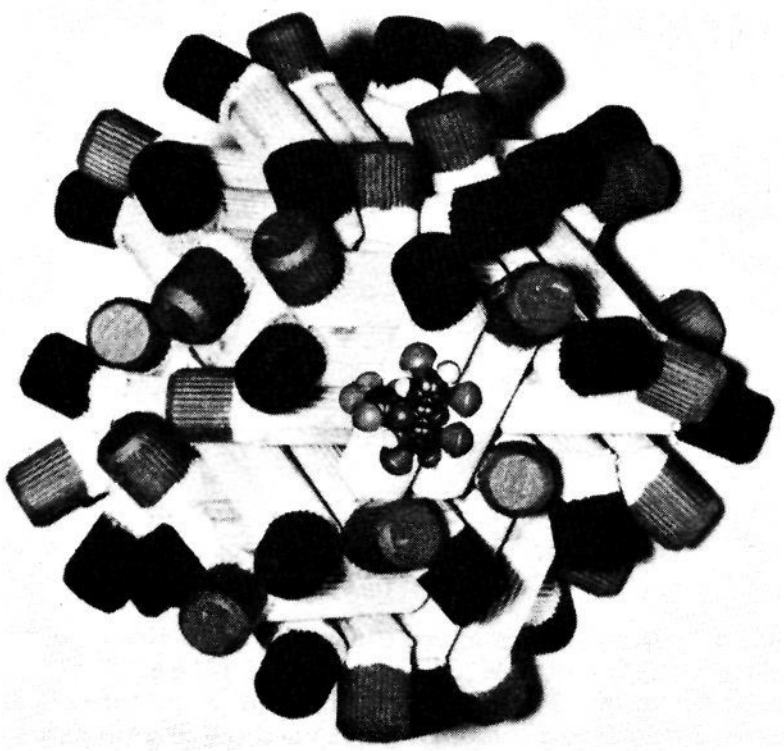

Figure 2. An oversimplified view of the interaction of rose bengal with aqueous micelles. The model is taken from ref 8.[26]

drawn for other rotators in aqueous micelles.[17] If isotropic diffusion and stick boundary conditions are assumed the rotational correlation time is given by the Stokes–Einstein equation

$$\tau_R \equiv 1/(6D) = \eta V/kT \qquad (2)$$

where D is the diffusion coefficient, η is the viscosity of the medium, V is the volume of the rotating molecule, k is the Boltzmann constant, and T is the absolute temperature. Equation 2 allows the calculation of the volume of rose bengal from τ_R and η. Taking τ_R = 2540 ps in 3.0×10^{-2} M CTABr (Table I) and η = 6 cP (extrapolated from a linear plot of τ_R vs. η, determined for rose bengal in various alcohols[14]) R = 7.4 Å and V = 1680 Å^3 are calculated for the radius and volume of rose bengal rotating in the micelle. The excellent agreement of this value with that given in EtOH (V = 1670 Å^3)[14] further supports the proposed mode of rotation (case b) and substantiates the assumed stick-boundary condition.

A second longer lived anisotropy decay of rose bengal was observable in micellar CTABr (see for example Figure 1C). Since this decay is substantially longer than the fluorescence lifetime of rose bengal, τ_R values associated with it can only be approximated. The mean of 8 separate runs for CTABr is 10.6 ± 2.0 ns, and within this experimental error, it is independent of the temperature. This value is most likely to be associated with rotation of the entire micelle (case c). It gives, in conjunction with η = 1.0 (for water), R = 22 ± 5 Å and V = 44 000 Å^3 which approximate the dimensions of the CTABr micelle (R = 35 Å).[23] It should be noted that the emission anisotropy does not approach zero within the time available for its measurements (Figure 1). This, together with the observed second exponential, suggests that rose bengal may only rotate about one of its axes when on the micellar surface, and hence complete depolarization requires the subsequent rotation of the micelle. A similar situation has been encountered for the rotation of diphenylhexatriene in liposomes.[24]

Partitioning of rose bengal in anionic SDS is much less favorable than cationic CTABr. This is manifested in shorter fluorescence lifetimes[25] and greater temperature dependence of τ_R than that observed for CTABr (Table I.). Indeed at 55 °C, τ_R could barely be observed.

The effective incorporation of rose bengal in SDS as well as the lack of appreciable pH effects on τ and τ_R in CTABr (Table I) points to the predominance of hydrophobic interactions. Rose bengal is likely to be "pulled" more strongly in the CTABr than in the SDS micelle as a result of both hydrophobic and electrostatic interactions. This is reflected in longer τ and τ_R, for rose bengal in CTABr, as opposed to SDS (Table I) where hydrophobic forces tend to drag in the probe. Rose bengal is relatively free to rotate in both micelles and experiences an environment similar to alcohol. The Hartley micelle would require the alignment of the rigid rose bengal molecule parallel to the hydrocarbon chain of the surfactant, and concomitant "deep penetration" (consider the radii: 7 Å for rose bengal, 20 Å for SDS, 35 Å for CTABr![23]). Such a solubilization would inevitably lead to the immobilization of the probe and rotational correlation times *entirely* governed by the tumbling of the micelle (case c). Assuming extensive exposure of surfactant hydrocarbons to water is the only way to rationalize the present data. Rose bengal is likely to rotate, relatively unhindered, on the micellar surface where water molecules are hydrating, and in contact with, a fair number of methylene groups. Placing rose bengal on the surfactant block model (Figure 2) provides perhaps the best visualization of the proposed mode of interaction.[26]

Acknowledgment. Support of the National Science Foundation is gratefully acknowledged. We are grateful to Dr. Michael Rodgers for providing information prior to publication. The competent technical help of David Danzeiser has appreciably aided this work.

(26) We are grateful to Dr. Fromherz for sending us photocopies of his model.

Fluoromethylglyoxal: Synthesis and Glyoxalase I Catalyzed Product Partitioning via a Presumed Enediol Intermediate

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Glyoxalase I [*S*-lactoylglutathione methylglyoxal-lyase (isomerizing) EC 4.4.1.5] catalyzes the conversion of the thiohemiacetal **2** of methylglyoxal (**1**) and glutathione (GSH) to the thioester **4** of D-lactic acid and GSH (Scheme I). Studies by Hall, Doweyko, and Jordan[1] have provided evidence for the intermediacy of the enediol **3** initially proposed by Racker[2] and a fast-shielded proton transfer to afford **4** with low incorporation of solvent protons into the C-2 position of the lactoyl moiety. Very recently, Shinkai et al.[3] have demonstrated that 3-methyltetra-*O*-acetylriboflavin inhibits glyoxalase I, presumably by oxidation of the transient enediol intermediate. This is in contrast to the intramolecular hydride shift mechanism proposed by other investigators.[4] We wish to report the synthesis of fluoromethylglyoxal (**5**), a new probe for this enzyme, and our finding that glyoxalase I catalyzes the partitioning of **5** and GSH into *S*-fluorolactoylglutathione and *S*-pyruvylglutathione with concomitant fluoride elimination. These results are consistent with the rapid proton-transfer mechanism and constitute the first use of a fluorinated substrate analogue to evaluate product partitioning via an enediol intermediate.

Fluoromethylglyoxal (**5**) was synthesized according to Scheme II. The dimethylketal **6** of fluorohydroxyacetone was prepared by a modification of the procedure of Pero et al.[5] Moffatt oxidation of **6** (dicyclohexylcarbodiimide/dimethyl sulfoxide/pyridinium trifluoroacetate) in ethyl ether afforded the dimethyl ketal of fluoromethylglyoxal (**7**) in 85% yield.[6] Deketalization

(1) (a) Hall, S. S.; Doweyko, A. M.; Jordan, F. *J. Am. Chem. Soc.* **1976**, *98*, 7460. (b) *Ibid.* **1978**, *100*, 5934.
(2) Racker, E. *J. Biol. Chem.* **1951**, *190*, 685.
(3) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *J. Am. Chem. Soc.* **1981**, *103*, 2070.
(4) (a) Rose, I. A. *Biochim. Biophys. Acta* **1957**, *25*, 214. (b) Franzen, V. *Chem. Ber.* **1956**, *89*, 1020.
(5) Pero, R. W.; Babiarz-Tracy, P.; Fondy, T. P. *J. Med. Chem.* **1977**, *20*, 644.

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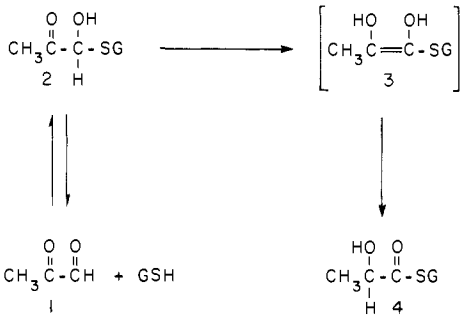
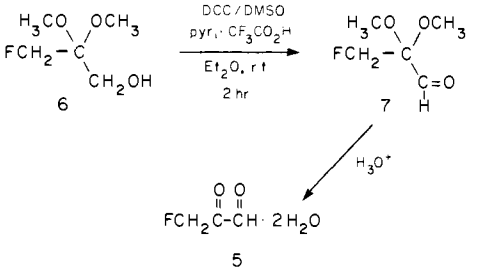
Scheme I



Scheme II



of **7** in 0.5 N HCl gave the title compound **5** in quantitative yield as the dihydrate.[7]

Incubation of **5** with glutathione in the presence of glyoxalase I (yeast; Sigma Grade X) resulted in the time-dependent formation of UV absorbing product(s) (λ_{max} 235 nm) as determined spectrophotometrically.[8] The product(s) generated were rapidly hydrolyzed upon addition of glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase, EC 3.1.2.6) (yeast, Sigma), thus establishing their structure(s) as thioesters.

The elimination of fluoride during the enzymatic reaction was monitored by using a fluoride ion electrode (Orion model 96–09) as shown in Figure 1. Incubation of **5** with glutathione in the presence of varying amounts of glyoxalase I (closed figures) resulted in the total release of fluoride.[9] The remarkable feature of this elimination was that its time course extended well beyond the time for complete thioester formation. For example, 6 units of glyoxalase I effected total fluoride release from **5** (0.5 mM) in ~2 h, while thioester product formation, measured spectrophotometrically, was complete in ~15 min (pH 6.6). Moreover, at low enzyme concentrations (e.g., 0.5 units), the kinetics was clearly biphasic, indicating a rapid release of a portion of the fluoride followed by a slower elimination to completion. In the presence of glyoxalase I and glyoxalase II, a single burst of fluoride release (open figures; Figure 1) was detected which followed a time dependence identical with thioester formation measured in the absence of glyoxalase II.[10] The ratio of fluoride released to total fluoride under these conditions was 0.32. This ratio was unaffected by glyoxalase I concentration, pH 5.5–7.5, and varying concentrations of **5** and glutathione.[11] When the burst of fluoride

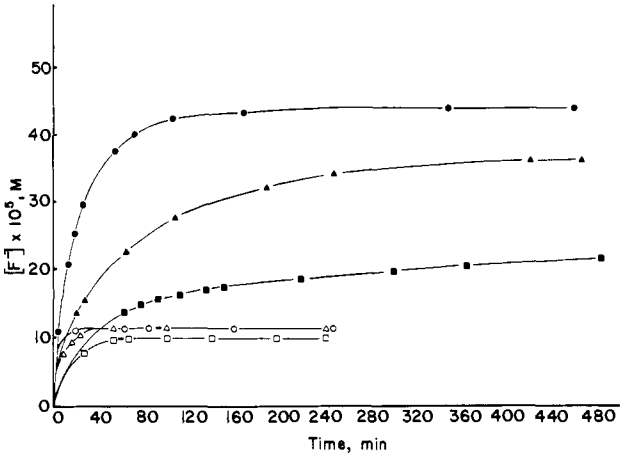


Figure 1. Glyoxalase I catalyzed elimination of fluoride from fluoromethylglyoxal (**5**) in 50 mM KPO_4, 2 mM GSH, pH 6.6, 25 °C. Closed figures: 6 units (●), 2 units (▲), and 0.5 unit (■) of glyoxalase I. Open figures: 6 units (○), 2 units (△), and 0.5 unit (□) of glyoxalase I and 0.5 unit of glyoxalase II. Early time points have been omitted for clarity.

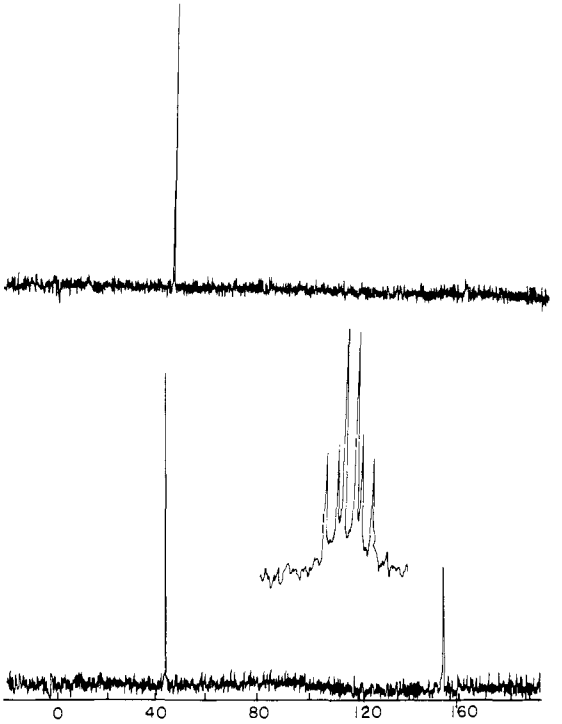


Figure 2. ^{19}F NMR (proton coupled) of the products from the reaction of fluoromethylglyoxal (**5**) with GSH and glyoxalase I in the absence (top) and presence (bottom) of glyoxalase II. Expanded spectrum of the fluorolactate multiplet in bottom spectrum is also shown (inset). Chemical shifts are relative to a 85% trifluoroacetic acid standard.

(6) Aqueous workup of the oxidation mixture was omitted in order to prevent hydration of the aldehyde which would complicate purification and analysis. Compound **7** was isolated by direct vacuum distillation from the reaction mixture (25 °C, 1 mmHg). **7**: ^1H NMR ($CDCl_3$) δ 9.60 (s, 1 H), 4.50 (d, 2 H, J = 47 Hz), 3.37 (s, 6 H); ^1H NMR (D_2O:Me_4Si external standard) δ 5.07 (d, 1 H, J = 2 Hz), 4.50 (d, 2 H, J = 47 Hz), 3.27 (s, 6 H); IR (neat) 1745 cm^{-1} (s); CI mass spectrum m/e 137 (MH^+), 105 ($MH^+ - CH_3OH$), 77 ($MH^+ - CH_3OH - CO$). The upfield shift of the aldehydic proton in D_2O indicates virtually complete hydration of the carbonyl moiety.

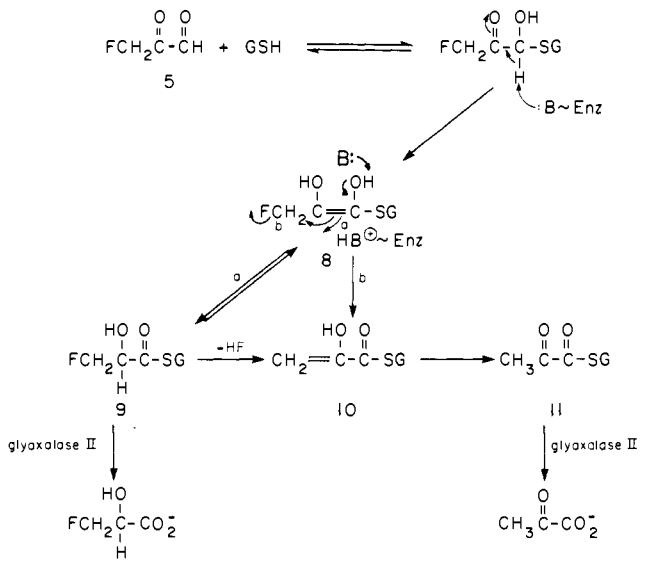
(7) Deketalization may be monitored by the ^1H NMR shift in the fluoromethyl protons or the methoxy protons as methanol is generated. At room temperature complete conversion takes several days. **5**: ^1H NMR (DCl/D_2O) 4.95 (d, 1 H, J = 2 Hz), 4.36 (d, 2 H, J = 47 Hz), 3.23 (s, 6 H, methanol); ^{19}F NMR (DCl/D_2O; 85% trifluoroacetic acid standard) −157 ppm (t, J = 47 Hz). Fluoromethylglyoxal is stable for months in aqueous HCl under N_2 and virtually no exchange of the fluoromethyl protons with solvent is observed. The absence of any fluoromethyl proton resonances in the region of ~5.5 ppm suggests that the keto moiety is also highly hydrated. In contrast, the ketone of methylglyoxal is ~33% hydrated under similar conditions. This is consistent with earlier observations that, while the keto group of pyruvate is 10–24% hydrated, the hydrate composition of fluoropyruvate is 85–95%. (a) Kokesh, F. C. *J. Org. Chem.* **1976**, *41*, 3593. (b) Goldstein, J. A.; Cheung, Y.-F.; Marletta, M. A.; Walsh, C. *Biochemistry* **1978**, *17*, 5567.

(8) Thioester formation was measured by the change of absorbance at 240 nm according to the method of Racker. When performed under identical conditions where the nonenzymatic formation of the thiohemiacetals was rate determining, fluoromethylglyoxal (**5**) was converted to thioester product(s) at ~$^1/_3$ the rate of methylglyoxal. This difference may be due in part to the different rates of dehydration of the keto aldehydes.

(9) The amount of fluoride released was in agreement with the amount of **5** added to the reaction mixture and the total amount of thioesters found assuming $\epsilon \simeq 3300$ at 240 nm for these products.

(10) The amount of glyoxalase II (0.5 units/mL) was sufficient to prevent the buildup of any detectable thioester. Higher concentrations of glyoxalase II afforded similar results.

Scheme III



elimination is subtracted from the kinetics of total release in the absence of glyoxalase II, the remaining fluoride elimination is sigmoidal, implicating its dependence upon the accumulation of thioester.[12]

The identity of fluoride-containing products was established by ^{19}F NMR spectroscopy (Figure 2). The glyoxalase I only reaction gave a sharp singlet for fluoride ion as expected. The glyoxalase I and II reaction afforded, in addition to the fluoride ion singlet, a proton-coupled sextet ($J_{2H,F}$ = 47, $J_{H,F}$ = 30 Hz) identical with authentic fluorolactate. This product eluted with fluorolactate from a Dowex 1 (formate; 0 → 6 M formic acid) column, and enzymatic and ORD analysis[13] suggested that it was predominantly (at least) the D isomer.

The unique glyoxalase I catalyzed partitioning of **5** is most simply explained by a partitioning of the enediol intermediate **8** between protonation to yield *S*-fluorolactoylglutathione (**9**) (path a) and elimination to form initially the enol (**10**) of *S*-pyruvylglutathione (**11**) (path b), which could tautomerize to **11** (Scheme III). Hydrolysis of the thioesters by glyoxalase II would yield pyruvate and fluorolactate which is inert to further elimination. In the absence of glyoxalase II the initially formed *S*-fluorolactoylglutathione (**9**) is susceptible to enzyme-catalyzed elimination due to backreaction as well as to chemical β elimination of fluoride. Quantitation of pyruvate formed by NADH and L-lactate dehydrogenase (L-lactate: NAD oxidoreductase EC 1.1.1.27) has verified that it is essentially identical with the amount of fluoride released under both enzyme reaction conditions. No inactivation of the yeast glyoxalase I was observed after as many as 2000 substrate turnovers.[14]

This is to our knowledge the first example of product partitioning of a fluorinated substrate.[15] Moreover, it provides strong support for the enediol intermediate since the elimination of fluoride is best accounted for by carbanionic character at C-2.[16]

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(11) Interestingly, our recent results demonstrate that this partition ratio is species dependent. The enzyme from rat erythocyte gives a ratio of 0.08, while the ratio for the mouse liver enzyme is 0.26.

(12) This point is also established by addition of glyoxalase II at any time during the glyoxalase-I-only elimination. This results in the immediate cessation of any further fluoride release.

(13) Craig, J. C.; Dummel, R. J.; Kun, E.; Roy, S. K. *Biochemistry* **1965**, *4*, 2547.

(14) Enzyme inactivation could conceivably have occurred via Michael addition of an active site nucleophile to the enol **10** prior to tautomerization to **11**:

$$H_2C{=}C(OH)C(=O)SG \xrightarrow{\text{Enz Nuc:}} CH_3C(=O)C(=O)SG + \text{Enz-Nuc-}CH_2C(H)(OH)C(=O)SG$$

Since **10** is at once an enol and an α,β-unsaturated thioester, this possibility is intriguing. That no inactivation is observed suggests that either **10** is not sufficiently activated for Michael addition or that no active site base is available. We are currently attempting to trap **10** in solution via nucleophilic attack. Kuo et al. (Kuo, D. J.; O'Connell, E. L.; Rose, I. A. *J. Am. Chem. Soc.* **1979**, *101*, 5025) have demonstrated that the enol of pyruvate has a $t_{1/2}$ for tautomerization of 30 s at pH 7.0. This supports the idea that **10** might be sufficiently long lived for Michael addition to occur.

(15) A large number of fluorinated substrate analogues have been designed for pyridoxal-dependent enzymes where the rate-determining step is usually Schiff's base hydrolysis. It is not surpising, therefore, that total fluoride elimination is observed. In contrast, the reaction of β-fluoroalanine with D-amino acid oxidase which presumably involves an α carbanionic intermediate resulted in the exclusive formation of fluoropyruvate (i.e., no elimination): Dang, T.-Y.; Cheung, Y.-F.; Walsh, C. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 960. Clearly, fluoromethylglyoxal constitutes a unique intermediary case.

(16) Preliminary studies indicate that chloro- and bromomethylglyoxal give exclusively elimination (i.e., pyruvate formation). This is consistent with their superior leaving group abilities (1:0.02:0.001 Br-Cl-F): Kosower, E. M. In "Physical Organic Chemistry"; Wiley: New York, 1968; p 81. An argument can be made for the possibility of a hydride transfer mechanism to afford **9** directly followed by a "fortuitous" enzyme-catalyzed elimination of fluoride. In this case, the partitioning observed could be due to competition of elimination with product release from the enzyme. Indeed, this sequence of events could also be used to explain the low amounts of solvent incorporation observed previously[1a] or the observations in this paper. Using substrate deuterated at C-1, we have recently observed an increase in the partitioning reaction (from 0.32 to 0.41). This effect is explainable by a selective primary isotope effect on the formation of **9** relative to fluoride elimination resulting in an overall increase in fluoride release. A hydride transfer, base-catalyzed elmination mechanism would have yielded a decrease in fluoride partitioning. Details of these findings will be published elsewhere.

Dicoordinated 2*H*-Phospholes as Transient Intermediates in the Reactions of Tervalent Phospholes at High Temperature. One-Step Synthesis of 1-Phosphanorbornadienes and Phosphorins from Phospholes

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We have recently shown that conveniently substituted λ^3 phospholes reacted easily with maleic anhydride and *N*-phenylmaleimide to give the expected [4 + 2] Diels-Alder cycloadduct.[1] The discovery that λ^3 phospholes could be rather reactive dienes prompted us to investigate their reactions with other dienophiles. Among acetylenic dienophiles, only the highly electrophilic dimethyl acetylenedicarboxylate has been reacted with phospholes previously.[2] The reactions were rather complicated but always started by an electrophilic attack on the phosphorus lone pair. We thus decided to investigate the reactions of the less electrophilic tolane with 1-phenyl-3,4-dimethylphosphole (**1**) and 1,2,5-triphenylphosphole (**2**).

At 170 °C, **1** reacted with tolane to give quite unexpectedly 3,4-dimethyl-2,5,6-triphenyl-1-phosphanorbornadiene (**3**) in quantitative yield.[3] The structure of **3** was unambiguously es-

(1) Mathey, F.; Mercier, F. *Tetrahedron Lett.* **1981**, *22*, 319.

(2) Hughes, A. N.; Uaboonkul, S. *Tetrahedron* **1968**, *24*, 3437. Waite, N. E.; Tebby, J. C. *J. Chem. Soc. C* **1970**, 386. Holah, D. G.; Hughes, A. N.; Kleemola, D.; *J. Heterocycl. Chem.* **1978**, *15*, 1319.

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