Synthesis and Properties of Halohydroxyacetones and Halomethylglyoxals

Ravi V. J. Chari and John W. Kozarich*

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510

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A short, high-yield synthetic scheme for the preparation of halohydroxyacetones (4) and halomethylglyoxals (6) from the corresponding 3-halopyruvic acids is described. The key intermediate to both classes is the dimethyl ketal (3) of the halohydroxyacetone (4). The ketal alcohol (3) may be hydrolyzed to 4 or oxidized to the corresponding aldehyde (5) which, in turn, may be hydrolyzed to the halomethylglyoxal (6). Halohydroxyacetones (4) exist in solution as an equilibrium mixture of the free ketone and hydrate. Halomethylglyoxals (6) occur nearly exclusively as the dihydrated species. Some other properties of these compounds are described.

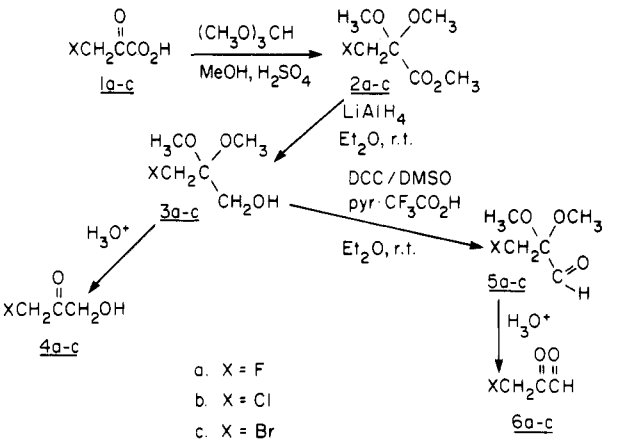
During our study of the mechanism of glyoxalase I,[1] we sought to develop a high-yield, efficient synthesis of halomethylglyoxals [3-halo-2-oxopropanals (6)] which would circumvent the problems of hydration of the carbonyl groups in synthetic intermediates, thereby complicating analysis, and of the sensitivity of α-keto aldehydes to alkaline conditions. We here report an abbreviated synthesis of the dimethyl ketal 3, its conversion to the corresponding halohydroxyacetones [3-halo-2-oxopropanols (4)] and halomethylglyoxals (6), and some of the properties of these compounds.

Results and Discussion

The previously reported synthesis[2] of 3 from the corresponding 3-halo-1,2-propanediol requires benzoylation of the primary hydroxyl group, Moffatt oxidation[3] of the secondary alcohol, ketalization of the carbonyl group, and alkaline hydrolysis of the benzoyl ester. Overall yield of the fluoro analogue (3a), for example, was ~28%. Our synthesis from the 3-halopyruvic acid (1) is outlined in Scheme I. The acids (1) were cleanly converted to the ketal esters (2) by reaction with trimethyl orthoformate and sulfuric acid in refluxing methanol, followed by aqueous workup and extraction with chloroform. The esters were smoothly reduced to the desired alcohols (3) with $LiAlH_4$ in diethyl ether. This reaction was particularly suited for the introduction of deuterium or tritium into the molecule via the appropriately labeled reducing agent and ultimately resulted in the specific labeling of the halomethylglyoxal (6) in the aldehydic position. The deuterated compound has been of considerable importance as a mechanistic probe for glyoxalase I.[4]

The dimethyl ketals (5) of the halomethylglyoxals were prepared by Moffatt oxidation[3] of the alcohols (3). Attempted oxidations by pyridinium chlorochromate,[5] chromium trioxide/pyrazole,[6] or cupric acetate,[7] which have been used with acid-sensitive compounds, proved unsuccessful, affording only starting material. In order to prevent hydration of the aldehydic group, aqueous workup of the reaction mixture was avoided. Instead, the aldehydes 5 were isolated by vacuum distillation from the reaction mixture. In this manner, anhydrous 5 may be obtained in high yield. The ketals 3 and 5 may be converted to the corresponding halohydroxyacetones (4) and halomethylglyoxals (6) by acid-catalyzed hydrolysis of the ketal. Since these compounds are optimally stable under acidic conditions, the advantage of the deketalizaton as the last step of the synthesis should be readily apparent.

Scheme I



Scheme II

Pero et al.[2] reported the deketalization of the fluoro alcohol (3a) to fluorohydroxyacetone (4a); however, no characterization of this compound was included. In order to establish the identity of the halohydroxyacetones, the deketalization of 3 was monitored by ^1H NMR in 1 N DCl in D_2O. A time course for the conversion of 3a to fluorohydroxyacetone (4a) is shown in Figure 1. Under these conditions, the fluoromethyl proton resonances centered at 4.40 ppm ($J_{H,F}$ = 47 Hz) exhibited a time-dependent decay with the concomitant appearance of two sets of fluoromethyl resonances centered at 5.1 and 4.3 ppm. Similarly, the hydroxymethyl proton doublet at 3.6 ppm ($J_{H,F}$ = 2 Hz) afforded a broad singlet at 4.3 ppm and a doublet at 3.5 ppm (J = 2 Hz), and a small shift in the methoxy resonance from 3.18 to 3.13 ppm was also observed since hydrolysis of the ketal gave methanol. Deketalization to 4a was essentially complete in 30 min. The NMR spectrum demonstrates that fluorohydroxyacetone exists as an equilibrium mixture of the free carbonyl and hydrated species (Scheme II). The upfield fluoromethyl and hydroxymethyl resonances are due to the hydrated species; the downfield resonances are thus originated from the free ketone. Peak integration suggests that under these conditions 4a was ~60% hydrated (K_{eq} = [hydrate]/[free carbonyl] = 1.5).

Deketalizations of the chloro (3b) and bromo (3c) analogues also yielded mixtures of the hydrated and free 4b

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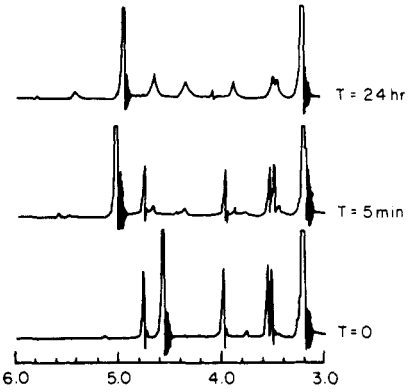


Figure 1. ^{1}H NMR (D_2O) time course of deketalization of **3a** to **4a** in 1 N DCl. Off scale peaks are due to HDO and methoxy (methanol) groups. T = 0: resonances at 4.8 and 4.0 ppm are due to fluoromethyl protons, and doublet at 3.6 ppm is due to hydroxymethyl protons. Spectrum was taken prior to DCl addition. T = 24 h: ketal is completely hydrolyzed. Resonance at 4.7 ppm results from overlap of fluoromethyl resonances of hydrated (also 3.9 ppm) and dehydrated (also 5.4 ppm) ketones. Hydroxymethyl resonances at 4.4 and 3.5 ppm are due to dehydrated and hydrated species, respectively.

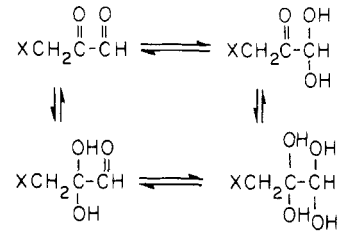
and **4c**, respectively. Chlorohydroxyacetone (**4b**) was ~42% hydrated (K_{eq} = 0.72), while bromohydroxyacetone (**4c**) was ~29% hydrated (K_{eq} = 0.41). This observation demonstrates that increased electron-withdrawing ability (F > Cl > Br) results in an increased destabilization of the carbonyl group relative to the hydrated species. The small shift in equilibrium of these two species in the homologous series suggests that the differences in the inductive effects are relatively minor. Halohydroxyacetones were found to be stable for months under argon in aqueous acid, exhibiting no loss of halide or exchange of the α protons. The absence of proton exchange suggests that these compounds do not readily undergo isomerization to the corresponding β-halo-α-hydroxypropionaldehyde via an enediol intermediate. Moreover, formation of an enediol could also have led to elimination of halide to ultimately yield methylglyoxal. Fluorohydroxyacetone (**4a**) was also stable in solution to pH 12, and the relative amounts of hydrate and free ketone appeared to be unaffected from pH 1 to 12. The chloro (**4b**) and bromo (**4c**) analogues were considerably labile under alkaline conditions presumably via nucleophilic substitution of the activated α-halo ketone by hydroxide or via intramolecular displacement by a hydrate oxygen anion to form an unstable epoxide.

The dimethyl ketals (**5**) were found to be strongly hydrated in aqueous solution. The anhydrous aldehydes afforded characteristic aldehydic proton resonances at 9.5–9.6 ppm in $CDCl_3$. In D_2O, no aldehyde resonances were detected and, instead, new resonances were observed at δ 5.1–5.2 [$CH(OH)_2$], indicating a virtually complete hydration of the aldehydes to the corresponding geminal diols.

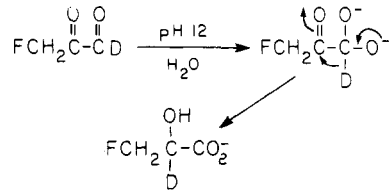
The dimethyl ketals (**5**) were also found to be remarkably stable toward acid-catalyzed deketalization. Under conditions (1.0 N HCl; room temperature) which resulted in the deketalization of the alcohols (**3**) in approximately 30 min, the aldehydes (**5**) required at least several days to be completely converted to the corresponding halomethylglyoxals (**6**). This suggests that the additional inductive effects of the aldehydic group (or *gem*-diol) relative to the alcohols (**3**) result in an increased activation energy for the deketalization, which proceeds via an oxonium ion intermediate.

^{1}H NMR analysis of the deketalization of **5** to **6** resulted in the direct formation of only one new set of halomethyl

Scheme III



Scheme IV



proton resonances which were slightly upfield in chemical shift to the starting ketals. Since deketalization to the unhydrated ketone group would have resulted in a considerable downfield shift (~1 ppm) in these resonances, these findings suggest that the ketone in the resulting halomethylglyoxals (**6**) is completely hydrated as well (Scheme III). In the case of methylglyoxal, NMR analysis has established that while the aldehyde is completely hydrated, the ketone is ~33% hydrated.[7] For the halomethylglyoxals, however, the molecule exists essentially exclusively as the dihydrate, since introduction of the halide results in an increased destabilization of the free ketone. Similar results have been reported for fluoropyruvic acid.[8]

The halomethylglyoxals (**6**) are stable for months in aqueous acid under argon but rapidly decompose in alkaline solutions. The observed products of alkaline treatment (pH 12) depend upon the halogen present. Fluoromethylglyoxal (**6a**) undergoes a rapid (~30 min) conversion to fluorolactate as determined by ^{19}F NMR (–151 ppm; D_2O, 85% trifluoroacetic acid standard; sextet, $J_{2H,F}$ = 47 Hz, $J_{H,F}$ = 30 Hz) and ion-exchange chromatography. That this reaction occurs via an internal Cannizaro reaction (1,2-hydride shift; Scheme IV) has been verified by analysis of the fluorolactate formed from fluoromethylglyoxal-d_1. ^{19}F NMR (–151 ppm, D_2O; triplet of triplets, $J_{2H,F}$ = 47 Hz, $J_{D,F}$ = 4.8 Hz) revealed that the deuterium was retained in the product, indicative of a hydride transfer mechanism. This is in marked contrast to the enzymatic conversion of fluoromethylglyoxal by the action of glyoxalases I and II and glutathione. This reaction has been shown to proceed via a proton transfer–enediol mechanism with retention of the aldehydic proton (deuteron) and partial retention of the fluoride.[4]

In the case of chloromethylglyoxal (**6b**) or bromomethylglyoxal (**6c**), glyceric acid is the observed product. The high reactivity of α-chloro and α-bromo ketones toward nucleophilic displacement (inter- or intramolecular as discussed above) suggests that **6b** and **6c** are rapidly converted in alkali to hydroxymethylglyoxal (hydroxypyruvaldehyde), which undergoes a 1,2-hydride shift to afford glycerate.

In conclusion, the syntheses of halohydroxyacetones (**4**) and halomethylglyoxals (**6**) reported here permit their preparation in large amounts and should lead to the fur-

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ther investigation of the chemistry of these compounds.

Experimental Section

Proton magnetic resonance spectra were recorded on a Varian Model T-60 spectrometer. ^{19}F NMR were recorded on a Bruker CXP 200 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Model 257 spectrometer. Mass spectra were recorded on a Hewlett Packard Model 5985 gas chromatograph/mass spectrometer.

Column chromatography was performed on Merck silica gel 60. 3-Fluoro- and 3-bromopyruvic acid were products of Sigma Chemical Co., St. Louis, MO. 3-Chloropyruvic acid was prepared by the literature method.[9] Lithium aluminum deuteride (98% D) was obtained from Aldrich Chemical Co., Milwaukee, WI. All other chemicals used were reagent grade. Elemental analyses were performed by Atlantic Micro Labs, Atlanta, GA.

General Procedure for the Preparation of Methyl 2,2-Dimethoxy-3-halopropanoates (2). A solution of the 3-halopyruvic acid (1; 25 mmol) in anhydrous methanol (50 mL) was treated with trimethyl orthoformate (15 mL) and concentrated H_2SO_4 (1.5 mL) and refluxed for 48 h. After cooling to room temperature, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (150 mL). The solution was extracted with $CHCl_3$ (3 × 50 mL), and the combined $CHCl_3$ extracts were washed with H_2O (100 mL) and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave the ketal esters 2 as colorless oils, which were further purified by distillation under reduced pressure to give analytical samples.

Methyl 2,2-dimethoxy-3-fluoropropanoate (2a): yield 59%; bp 24–5 °C (0.2 mm); IR (neat) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (d, 2 H, J = 47 Hz), 3.73 (s, 3 H), 3.23 (s, 6 H); EI mass spectrum, m/e (relative intensity) 135 ($M^+ - OCH_3$, 20), 133 ($M^+ - FCH_2$, 10), 107 ($M^+ - CO_2CH_3$, 100), 59 [$M^+ - FCH_2C(OMe)_2$, 40].

Anal. Calcd for $C_6H_{11}FO_4$: C, 43.37; H, 6.67. Found: C, 42.81; H, 6.74.

Methyl 3-chloro-2,2-dimethoxypropanoate (2b): yield 76%; bp 83–5 °C (20 mm); IR (neat) 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.87 (s, 3 H), 3.80 (s, 2 H) 3.35 (s, 6 H).

Anal. Calcd for $C_6H_{11}ClO_4$: C, 39.46; H, 6.07; Cl, 19.41. Found: C, 39.45; H, 6.07; Cl, 19.37.

Methyl 3-bromo-2,2-dimethoxypropanoate (2c): yield 81%: bp 42–3 °C (0.5 mm); IR (neat) 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.73 (s, 3 H), 3.53 (s, 2 H) 3.23 (s, 6 H); EI mass spectrum, m/e (relative intensity) 195, 197 ($M^+ - OCH_3$, 10) 167, 169 ($M^+ - CO_2CH_3$, 100), 133 ($M^+ - BrCH_2$, 35), 93, 95 [$M^+ - C(OMe)_2 - CO_2Me$, 12).

Anal. Calcd for $C_6H_{11}BrO_4$: C, 31.74; H, 4.88; Br, 35.19; Found: C, 31.81; H, 4.91; Br, 35.10.

$LiAlH_4$ Reduction of Methyl 2,2-Dimethoxy-3-halopropanoates (2). A suspension of $LiAlH_4$ (or $LiAlD_4$, 9.6 mmol) in anhydrous ether (30 mL) was cooled in ice, and a solution of the ester 2 (8 mmol) in anhydrous ether (10 mL) was added dropwise with stirring over a period of 10 min. The reaction mixture was then stirred at room temperature for 2 h. The excess $LiAlH_4$ was destroyed by addition of ethyl acetate (5 mL). H_2O (30 mL) was added, and the pH of the solution was adjusted to 3.0 by addition of 1 N HCl, followed by rapid neutralization with a saturated solution of $NaHCO_3$. The aqueous mixture was extracted with ether (3 × 50 mL). The combined ether extracts were washed with saturated solutions of $NaHCO_3$ (50 mL) and H_2O (100 mL) and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave pure 3-halo-2,2-dimethoxy-1-propanol (3) as colorless oils. Analytical samples were obtained by distillation of the product under vacuum.

2,2-Dimethoxy-3-fluoro-1-propanol (3a): yield 89%; bp 30–32 °C (0.5 mm) [lit.[1] bp 35 °C (0.25 mm)]; IR (neat) 3500–3380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.40 (d, 2 H, J = 47 Hz, FCH_2), 3.63 (d, 2 H, J = 2 Hz, CH_2OH), 3.27 (s, 6 H, OCH_3), 2.32 (s, 1 H, OH).

Anal. Calcd for $C_5H_{11}FO_3$: C, 43.47; H, 8.04. Found: C, 43.43; H, 8.05.

2,2-Dimethoxy-3-fluoro-1-propanol-*1,1*-d_2 (3a): yield 85%; bp 35–36 °C (0.5 mm); IR (neat) 3500–3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.38 (d, 2 H, J = 47 Hz), 3.25 (s, 6 H), 2.65 (s, 1 H); EI mass spectrum, m/e (relative intensity) 109 ($M^+ - OCH_3$, 43), 107 ($M^+ - CD_2OH$, 100), 77 ($M^+ - CD_2O - OCH_3$, 33).

3-Chloro-2,2-dimethoxy-1-propanol (3b): yield 95%; bp 51–53 °C (0.5 mm); IR (neat) 3500–3420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.62 (s, 2 H), 3.53 (s, 2 H), 3.20 (s, 6 H), 2.97 (br s, 1 H).

Anal. Calcd for $C_5H_{11}ClO_3$: C, 38.85; H, 7.17; Cl, 22.93. Found: C, 38.68; H, 7.21; Cl, 22.84.

3-Bromo-2,2-dimethoxy-1-propanol (3c): yield 86%; decomposed on attempted vacuum distillation under an argon atmosphere; IR (neat) 3500–3400 cm^{-1}; NMR ($CDCl_3$) δ 3.67 (s, 2 H), 3.42 (s, 2 H), 3.20 (s, 6 H), 2.53 (s, 1 H); EI mass spectrum, m/e (relative intensity) 167, 169 ($M^+ - OCH_3$, 100), 135, 137 ($M^+ - OCH_3 - CH_3OH$, 40), 105 ($M^+ - BrCH_2$, 62), 93, 95 [$M^+ - C(OMe)_2 - CH_2OH$, 62).

3-Halo-1-hydroxypropan-2-one (halohydroxyacetone, 4) was prepared by deketalization of 3 in aqueous acid. A solution of 2,2-dimethoxy-3-halo-1-propanol (3; 0.70 mmol) in D_2O (0.5 mL) was placed in an NMR tube. DCl, 10 N, was added to a final concentration of 1 N. ^1H NMR spectra were recorded at time intervals of T = 0 (before DCl addition), 5, 30 min, and 24 h at room temperature. Deketalization was complete in 30 min as evidenced by the disappearance of the OCH_3 (ketal) peak and appearance of a new peak for OCH_3 (methanol).

3-Fluoro-1-hydroxypropan-2-one (fluorohydroxyacetone, 4a): ^1H NMR (D_2O; Me_4Si external standard, T = 0) δ 4.37 (d, 2 H, J = 47 Hz), 3.53 (d, 2 H, J = 2 Hz), 3.19 (s, 6 H); NMR (D_2O + 1 N DCl, T = 5 min) δ 5.12 (d, J = 47 Hz) and 4.33 (d, J = 47 Hz) (total 2 H), 4.31 (br s) and 3.48 (d, J = 47 Hz) (total 2 H), 3.18 (s) and 3.13 (s) (total 6 H); ^1H NMR (D_2O and 1 N DCl, T = 24 h) δ 4.96 (d, J = 47 Hz) and 4.17 (d, J = 47 Hz) (total 2 H, FCH_2), 4.27 (d, J = 1 Hz) and 3.38 (d, J = 2 Hz) (total 2 H), 3.13 (s, 6 H, methanol).

3-Chloro-1-hydroxypropan-2-one (chlorohydroxyacetone, 4b): ^1H NMR (D_2O; Me_4Si external standard, T = 0) δ 3.73 (s, 4 H), 3.33 (s, 6 H); NMR (D_2O + DCl, T = 24 h) δ 4.37 (s) and 3.53 (s) (total 2 H), 4.33 (s) and 3.50 (s) (total 2 H), 3.22 (s, 6 H, methanol).

3-Bromo-1-hydroxypropan-2-one (bromohydroxyacetone, 4c): ^1H NMR (D_2O; Me_4Si external standard, T = 0) δ 3.85 (s, 2 H), 3.68 (s, 2 H), 3.40 (s, 6 H); NMR (D_2O + DCl, T = 24 h), 4.60 (s), 3.70 (br s), 4.27 (s), 3.70 (br s) (total 4 H), 3.36 (s, 6 H).

2,2-Dimethyl-3-halo-1-propanal (5). A solution of the alcohol 3 (5.0 mmol) in anhydrous ether (10 mL) was treated with *N,N'*-dicyclohexylcarbodiimide (3.10 g, 15 mmol), anhydrous Me_2SO (0.39 mL, 5.5 mmol) and anhydrous pyridine (0.20 mL, 2.5 mmol). The reaction flask was equipped with a drying tube, and the contents were stirred and cooled in ice. Trifluoroacetic acid (0.19 mL, 2.4 mmol) was added, and the reaction mixture was stirred for 10 min at 0 °C and then for an additional period of 2 h at room temperature. The precipitate was filtered off, and the solvent was evaporated under reduced pressure. Distillation of the residue under vacuum gave 3-halo-2,2-dimethoxy-1-propanal (5) as a colorless oil.

2,2-Dimethoxy-3-fluoro-1-propanal (5a): yield 81%; bp 25–27 °C (1 mm); IR (neat) 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.60 (s, 1 H), 4.50 (d, 2 H, J = 48 Hz), 3.37 (s, 6 H); CI mass spectrum, m/e 137 (MH^+), 106 ($MH^+ - OCH_3$), 105 ($MH^+ - CH_3OH$), 77 ($MH^+ - OCH_3 - CHO$).

2,2-Dimethoxy-3-fluoro-1-propanal-*1*-d_1: yield 87%; bp 25–27 °C (0.5 mm); IR (neat) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (d, 2 H, J = 47 Hz) and 3.33 (s, 6 H); ^1H NMR (D_2O; Me_4Si external standard) δ 4.50 (d, 2 H, J = 47 Hz) and 3.27 (s, 6 H); EI mass spectrum, m/e (relative intensity) 107 ($M^+ - CDO$, 100), 106 ($M^+ - OCH_3$, 30), 104 ($M^+ - FCH_2$, 5), 73 ($M^+ - OCH_3 - FCH_2$, 25).

3-Chloro-2,2-dimethoxy-1-propanal: yield 77%; bp 24–26 °C (0.5 mm). The product which had traces of Me_2SO was further purified by column chromatography on silica gel eluting with $CHCl_3$ to give the pure aldehyde **5b**, which was redistilled to give an analytical sample: IR (neat) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.60 (s, 1 H), 3.63 (s, 2 H), 3.27 (s, 6 H).

Anal. Calcd for $C_5H_9ClO_3$: C, 39.36; H, 5.95; Cl, 23.24. Found: C, 39.29; H, 5.97; Cl, 23.22.

3-Bromo-2,2-dimethoxy-1-propanal (5c): yield 86%; bp 30–32 °C (0.2 mm). The product which had traces of Me_2SO was

(9) E. J. Cragoe and C. R. Robb, *Org. Synth.*, **40**, 54 (1960).

further purified by column chromatography on silica gel eluting with $CHCl_3$ to give the pure aldehyde **5c**, which was redistilled to give an analytical sample: IR (neat) 1740 cm^{-1}; NMR ($CDCl_3$) δ 9.50 (s, 1 H), 3.47 (s, 2 H), 3.30 (s, 6 H).

Anal. Calcd for $C_5H_9BrO_3$: C, 30.48; H, 4.60; Br, 40.55. Found: C, 30.42; H, 4.64; Br, 40.49.

3-Halo-2-oxopropanal (Halomethylglyoxal, 6). A solution of 2,2-dimethoxy-3-halo-1-propanal (**5**; 0.7 mmol) in D_2O (0.5 mL) was placed in an NMR tube. DCl, 10 N, was added to a final concentration of 1 N. ^1H NMR spectra were recorded at time intervals of $T = 0$ (no DCl), 1 h, 24 h, 72 h, and 1 week at room temperature. The extent of deketalization was determined by comparing the ratios of the OMe (ketal) to the OMe (methanol) peak and was found to be complete after 1 week to give 3-halo-2-oxopropanal (halomethylglyoxal, **6**).

Fluoromethylglyoxal (6a): ^1H NMR (D_2O; Me_4Si external standard, $T = 0$) δ 5.07 (d, 1 H, $J = 2$ Hz), 4.50 (d, 2 H, $J = 47$ Hz), 3.27 (s, 6 H); ^1H NMR (D_2O + 1 N DCl, $T = 1$ week) δ 4.95 (d, 1 H, $J = 2$ Hz), 4.36 (d, 2 H, $J = 47$ Hz), 3.23 (s, 6 H); ^{19}F NMR (H_2O) proton coupled, −156.855 ppm (t, $J = 47$ Hz).

Chloromethylglyoxal (6b): ^1H NMR (D_2O; Me_4Si external standard, $T = 0$) δ 5.03 (s, 1 H), 3.58 (s, 2 H), 3.20 (s, 6 H); NMR (D_2O + 1 N DCl, $T = 1$ week) δ 4.73 (s, 1 H), 3.43 (s, 2 H), 3.07 (s, 6 H).

Bromomethylglyoxal (6c): ^1H NMR (D_2O; Me_4Si external standard, $T = 0$) δ 5.20 (s, 1 H), 3.57 (s, 2 H), 3.30 (s, 6 H); NMR (D_2O + 1 N DCl, $T = 1$ week) δ 4.90 (s, partially masked by HDO), 3.43 (s, 2 H), 3.17 (s, 6 H).

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Registry No. **1a**, 433-48-7; **1b**, 3681-17-2; **1c**, 1113-59-3; **2a**, 81371-75-7; **2b**, 55900-23-7; **2c**, 81371-76-8; **3a**, 62741-32-6; **3a**-d_2, 81371-77-9; **3b**, 81371-78-0; **3c**, 81371-79-1; **4a**, 62522-70-7; **4b**, 24423-98-1; **4c**, 38987-72-3; **5a**, 78381-95-0; **5a**-d_1, 81371-80-4; **5b**, 81371-81-5; **5c**, 81371-82-6; **6a**, 78381-94-9; **6b**, 81371-83-7; **6c**, 81371-84-8.

Botryodiplodin, a Mycotoxin Synthesized by a Strain of *P. roqueforti*

S. Moreau,*† A. Lablache-Combier,‡ J. Biguet,† C. Foulon,§ and M. Delfosse§

INSERM, U-42, 59650 Villeneuve d'Ascq, France, Laboratoire de Chimie Organique Physique, Associé à l'ENSCL, ERA CNRS No. 827, Université des Sciences et Technique de Lille I, 59655 Villeneuve d'Ascq Cédex, France, and Laboratoire de Dynamique des Cristaux Moléculaires, ERA CNRS No. 456, Université des Sciences et Techniques de Lille I, 59655 Villeneuve d'Ascq Cédex, France

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A toxic strain of *Penicillium roqueforti* synthesized the botryodiplodin, a mycotoxin classically elaborated by the *Botryodiplodin theobromae* Pat., whose chemical structure and stereochemistry (obtained by X-ray analysis) are reported.

Toxic metabolites from *Penicillium roqueforti* have been reported by various authors. For example WEI[1] isolated and identified the PR toxin from culture media of the NRRL 849 *P. roqueforti* strain. From this and various other strains we have isolated closely related metabolites such as the eremofortins A–E.[2-4] Alkaloids such as roquefortine,[5] isofumigaclavine,[6] and marcfortine A[7] have also been isolated from the mycelium of this species. Significantly, Lafont[8] showed that the presence of PR toxin could not explain the toxicity of numerous strains of *P. roqueforti*. We have therefore turned our interest towards non-PR-toxin strains of this species, and in this paper we report the isolation and identification of a new toxin from *P. roqueforti*, botryodiplodin.

Results and Discussion

The examination of *P. roqueforti* isolated from various silages enabled us to detect non-PR-toxin producing strains. All were tested for toxicity on mice by intraperitoneal injections of crude chloroform extracts from cultured media. The toxic B-26 strain was chosen for identification of the toxic metabolites.

The chloroform extract of the culture medium was purified by chromatography over silica gel by using a stepwise gradient mixture of methanol–chloroform. The fraction elected by a 2/98 (v/v) mixture was revealed to be toxic.

Table I. Fractional Atomic Parameters

atom	x	y	z
O_1	0.7097	0.9293	−0.1521
O_2	0.4914	1.0112	0.1429
O_3	0.4024	0.8205	0.1466
O_4	0.2685	0.9182	0.2061
C_1	0.4218	0.9307	0.0183
C_2	0.4657	0.8774	−0.1555
C_3	0.5705	0.8541	−0.0310
C_4	0.5863	0.9621	0.1333
C_5	0.4601	0.9859	−0.3277
C_6	0.6455	0.8523	−0.1714
C_7	0.6346	0.7430	−0.3310
C_8	0.3243	0.8310	0.2333
C_9	0.3174	0.7108	0.3665

Further purification was achieved by HPLC. Crystallization from ethyl ether afforded a white crystalline sample of compound 1 (Figure 1).

† INSERM.
‡ Laboratoire de Chimie Organique Physique.
§ Laboratoire de Dynamique des Cristaux Moléculaires.

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