6-Methoxy-2-benzoxazolinone Synthesis in 1,3-Butanediol

John D. Richey, Albert L. Caskey and J. N. BeMiller

Department of Chemistry and Biochemistry
Southern Illinois University at Carbondale
Carbondale, Illinois 62901

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Synthesis of 6-methoxy-2-benzoxazolinone (MBOA) has been accomplished with a significantly improved yield by condensation of excess 2-hydroxy-4-methoxyphenylammonium chloride with urea in 1,3-butane diol solvent. The condensation occurs readily even in 50-mg or 100-mg batches enabling a convenient preparation of \([^{14}\text{C}]\) MBOA.

Several 2-(2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one) \(\beta\)-D-glucopyranosides are found in cereal plants. Living and healthy corn (Zea mays L.) and wheat plants contain 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) as a stable glucoside.\(^2\,3\) When tissue damage occurs, the glucoside is cleaved enzymically to the aglucone, DIMBOA, which subsequently is converted spontaneously into the antifungal 6-methoxy-2-benzoxazolinone (MBOA) \(^4\) 2,4-Dihydroxy-2H-1,4-benzoxazine-3(4H)-one glucoside also has been found in corn tissue,\(^6\) in this case, the labile aglucone decomposes to 2-benzoxazolinone. In addition, air-dried corn coleoptile tissue was observed to contain 6,7-dimethoxy-2-benzoxazolinone after heating.\(^7\) DIMBOA glucoside and MBOA concentrations have been related to corn-plant resistance to the European corn borer\(^8\) and to stalk rot.\(^9\)

A number of analytical procedures for the determination of 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one glucosides in plant tissues measure concentrations of 2-benzoxazolinones, evaluated as MBOA, in the final steps of the procedures. Thus, there is a need for accurate, rapid, and sensitive methods for the quantitative determination of MBOA if careful studies are to be made of the various factors (genetic background, growth time period, temperature, illumination received, and perhaps available iron) which affect apparent MBOA concentrations.\(^10\,11\) Such methods will require high-purity, primary-standard MBOA, the preparation of which demands a well defined synthetic route, that includes effective purifications of intermediates.

Scism \textit{et al.} recently reported a quantitative method in which the 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one glucosides in corn were cleaved enzymically, and the aglucones formed were converted, by hydrolysis in boiling water, to the more stable 2-benzoxazolinones, which subsequently were extracted and quantified as MBOA using absorbance at 55.5 \(\mu\)m in the infrared.\(^12\) This IR method required quantitative recovery through several steps of extraction and purification.

Klun and Brindley\(^13\) avoided the difficulty of requiring quantitative extraction by use of \([^{14}\text{C}]\) MBOA in a combined spectrophotometric-isotope-dilution method. MBOA in the final solution was quantified by spectrophotometric measurement at 230 nm, which enabled calculation first of the \([^{14}\text{C}]\text{MBOA}\) specific activity and then of the concentration of MBOA in the original sample. Spectrophotometric determinations on plant materials are complicated by the presence of interfering substances with broad absorption bands in the UV region; Klun and Brindley used three, different, consecutive, thin-layer-chromatographic separations to reduce the problem.\(^12\) Such time-consuming steps inspired Scism \textit{et al.} to use IR spectrophotometry for MBOA quantification.\(^12\) A method dependent only on extractions and filtrations accompanied by simple quantification and isotope-dilution measurements would
eliminate the interference problems of UV spectrophotometry and the tedious manipulation problems of quantitative IR spectrophotometry, and would lend itself toward rapid analysis of a large number of samples, but necessitates a high-yield, radiochemically pure, small-quantity synthesis of primary-standard-quality MBOA with a relatively high specific activity.

Report of the synthesis of MBOA in reasonably good yield was not given until Allen and Laird\(^\text{14}\) reported a synthesis of MBOA (overall yield \(\sim 17\%\) MBOA). Their method required a number of precautions such as the need to work in the dark or under photographic safety lights, use of anhydrous hydrogen chloride gas, maintenance of an inert atmosphere over solutions of certain intermediates, and avoidance of water during certain synthetic steps. Klun and Brindley\(^\text{13}\) did not report the yield for their MBOA-producing fusion with \([^{14}\text{C}]\text{urea}\).

This laboratory recently reported\(^\text{15}\) a simplified, more practical procedure for the production of 0.1-mole quantities of non-labeled MBOA. The synthesis commenced with the nitration, in glacial acetic acid, of 3-methoxyphenol (1) to form 5-methoxy-2-nitrophenol(2), which subsequently was reduced by sodium dithionite to 2-amino-5-methoxyphenol. The amine was treated with hydrochloric acid and converted to 2-hydroxy-4-methoxyphenylammonium chloride (3), which was fused with urea to yield MBOA (4). However, the fusion of 3 with urea was complicated by urea volatilization, and produced erratic yields in small-quantity fusions; 0.60 g urea was used as the basis of the smallest reactions since any smaller-sized reactions gave wildly varying results.

Subsequently, we investigated the problems related to production of \([^{14}\text{C}]\text{MBOA}\), which must be prepared in small quantities to keep the specific activity high and to maximize personnel safety without utilization of elaborate radiochemical-safety precautions. To stabilize yields, and thereby assure more efficient use and safer control of \([^{14}\text{C}]\text{urea}\), which would be volatilized during fusion with 3, the condensation of 3 to MBOA was attempted in a solvent; the attempts to stabilize yields by production of MBOA in solvent media were successful, and a significantly increased yield also was observed.

The selected solvent needed to be a good solvent for the reactants, 3 and urea; high boiling, in order to permit the condensation to occur at 180°C; not particularly reactive itself; water soluble in order to dissolve concentrated hydrochloric acid; and diethyl ether insoluble to permit extraction of MBOA. Several solvents were considered, including 3,5-dihydroxyaniline, mixed hexanetriols, 1,2-diamino-4,5-dimethoxybenzene, 1-amino-n-undecane, 4-hydroxypridazine, and 1,3-butanediol. 1, 3-Butanediol was selected on the basis of expected stability, liquid range, inertness to reaction, solubilities, and non-toxic, non-odorous properties. Urea and 3, were dissolved together in the previously dried solvent, and the resulting solution was heated at elevated temperatures for several hours. The solution was cooled and treated with excess concentrated hydrochloric acid to ensure that the excess amine present was converted to the hydrochloride, and the MBOA was extracted from the solution with immiscible diethyl ether. Reactants and product were weighed accurately. The stabilization of the yield was excellent (Table I).

The maximization of MBOA yield with respect to urea then was examined. Replicate,
accurately weighed samples of urea, 0.1000 g±0.0005 g, were combined with accurately weighed amounts of 3 (in 0.030 g increments from 0.280 g to 0.730 g, 1.62×10⁻³ mole to 4.16×10⁻³ mole; mole ratios urea/3 ranged from 1/0.97 to 1/2.5). Approximately 20 ml of 1,3-butanediol was added to each, and the solutions were reacted in identical manners. Some leveling of yields occurred as the mole fraction of 3 became larger (Fig. 1). The average yield of MBOA with respect to urea for reactions at mole ratios of 1/1.7 to 1/2.5 was 46% (standard deviation 4.9%), or 1.7 times larger than the best yield of MBOA with respect to urea for the fusion reaction.

The nitration of 1 in glacial acetic acid was observed earlier to be tedious because of considerable tar formation, and because of the need to steam distill 2 from the reaction mixture, which also required proper ventilation to eliminate physiological effects. It was found that addition of small amounts of toluene to the reaction mixture in the conversion of 1 to 2 greatly reduced the tar formation, and significantly reduced the difficulties associated with the purification of 2 from its reaction mixture by steam distillation. However, large amounts of toluene slowed the reaction and significantly reduced yields. When acetic anhydride was used to remove traces of water in the reaction mixture during formation of 2, the yield was decreased and tar formation was increased greatly.

The subsequent reduction of 2, and isolation of the product, typically yielded 5-methoxy-2-aminophenol in the form of a white, crystalline solid which became colored red after 1~2 days in light. However, reasonably large (2~5 mm) crystals of 5-methoxy-2-aminophenol were produced after the heating of 2 with sodium dithionite in an aqueous medium; the flask containing the reaction mixture was covered externally with aluminum foil, and the reaction mixture was allowed to cool to room temperature in the dark and remain overnight. Transparent, almost colorless but slightly reddish crystals of the amine formed overnight; their identity was confirmed by IR spectroscopy. The crystals turned red, and then an opaque, dark red upon illumination by overhead fluorescent lights in the laboratory. The IR spectra of the slightly colored, transparent crystals and the opaque, dark-red crystals were not noticeably different. Both colored forms of the amine, when reacted with aqueous HCl, formed the purple aqueous solutions and light-blue crystals of 3, and the different batches when dried and reacted with urea were indistinguishable in yield or quality of MBOA produced. This observation further demonstrates the insensitivity of this synthetic route to variations in various ambient conditions.

Table I. Stability of Yield of Condensation in 1,3-Butanediol

<table>
<thead>
<tr>
<th>RNH₂Cl (g)</th>
<th>Urea (g)</th>
<th>Yield of MBOA (g)</th>
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</thead>
<tbody>
<tr>
<td>0.050</td>
<td>0.050</td>
<td>0.0130 0.0120 0.0130</td>
</tr>
<tr>
<td>0.290</td>
<td>0.100</td>
<td>0.1033 0.0984 0.0957</td>
</tr>
<tr>
<td>0.350</td>
<td>0.100</td>
<td>0.1067 0.1063 0.1101</td>
</tr>
</tbody>
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a) 2-Hydroxy-4-methoxyphenylammonium chloride.

Fig. 1. Effect of Mole Ratio of Reactants on MBOA Yield.
The solid lines show ±1.0 standard deviation.
EXPERIMENTAL

Melting points were determined in open capillary tubes, using a calibrated Fisher Johns Melting Point Apparatus, but were not corrected.

5-Methoxy-2-nitrophenol (2)

3-Methoxyphenol (1) (49.1 g, 0.396 mole) was dissolved in glacial acetic acid (50 g) and toluene (3.0 ml), and was nitrated at 0°C by the gradual addition of a concentrated solution of HNO₃ (35 ml, 51 g, 0.567 mole) in glacial acetic acid (150 g). The clear, greenish mixture reacted evenly and mildly vigorously upon sitting approximately 30 seconds, and evolved nitric oxide. The brown solution was allowed to sit overnight and was steam distilled without difficulty in a hood, yielding in ~7 hr 17.6 g (26.3%) of 2.

6-Methoxy-2-benzoxazolinone (4)

2-Hydroxy-4-methoxyphenylammonium chloride (3) was prepared by a procedure previously reported by this laboratory.15) 3 (0.630 g, 3.59×10⁻³ mole) was placed into a large test tube with urea (0.1000±0.0005 g, 1.67×10⁻³ mole) and 20 ml of 1,3-butanediol (previously dried by heating to 180°C for 2 hr). The mixture was heated at 160~180°C for 2 hr, cooled, and acidified by the addition of 1:3 v/v concentrated HCl-water. The resulting solution was transferred to a separatory funnel and extracted three times with diethyl ether (50-ml portions). The three ether extracts were combined, and the ether was removed on a rotary evaporator under reduced pressure. The residue, 4, was transferred to a 100-ml beaker and recrystallized from water. The slightly reddish crystals were gathered, dried, and weighed (yield 46~56%): mp 152.5~154°C for all batches synthesized.

REFERENCES

1) To whom correspondence should be addressed.
12) A. J. Scism, J. N. BeMiller and A. L. Caskey, Anal. Biochem., 58, 1 (1974). The DIMBOA and MBOA reported by Scism et al. are identical to the respective substances reported herein, where current Chemical Abstracts nomenclature is used.