

5-, 6-, and 7-Methoxybenzoxazolinone: Carbon-13 Nuclear Magnetic Resonance Spectra and Biological Activity

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For elucidation of structure-activity relationships, 5-, 6-, and 7-methoxybenzoxazolinones were synthesized and found to have generally similar activities as larval growth inhibitors of the Southwestern corn borer (*Diatraea grandiosella*, Dyar), the fall armyworm (*Spodoptera frugiperda*, J. E. Smith), and the European corn borer (*Ostrinia nubilalis*, Hübner). The compounds were also evaluated for inhibition of radish (*Raphanus sativus*) and tomato (*Lycopersicon esculentum*) seedling growth and for antibacterial activity with *Pseudomonas maltophilia*. Assignments of ^{13}C magnetic resonances (^{13}C NMR) were made by using gated decoupling spectra.

6-Methoxybenzoxazolinone (6-MBOA) has been found in cereal (Virtanen and Hictala, 1955; Gahagan and Mumma, 1967; Venis and Watson, 1978) and in *Scopara dulcis* (Chen and Chen, 1976). It was reported as a host plant resistance factor to the European corn borer (*Ostrinia nubilalis* Hübner) (Klun and Robinson, 1969) and also as having hypotensive activity and antidiabetic potential (Venis and Watson, 1978).

Synthesis and ^1H NMR data have been reported for 6-MBOA by Matsumoto and Okazawa (1974), Richey, et al. (1976), and Chen and Chen (1976). The procedure of Matsumoto and Okazawa (1974) was used for our synthesis of 5-, 6-, and 7-MBOA (Figure 1).

We discuss the ^{13}C NMR spectra of these three compounds; the attribution of the different shifts to the individual carbons was achieved with gated decoupling used in conjunction with the pulsed FT (Fourier transformation) technique to retain the increase in signal to noise produced by the nuclear Overhauser effect (NOE) while at the same time giving a completely coupled spectrum.

The three isomers (5-, 6-, and 7-MBOA) were tested as growth inhibitors of radish and tomato seedlings, for antibacterial activity with *Pseudomonas maltophilia*, and for larval growth inhibition of the Southwestern corn borer (SWCB) (*Diatraea grandiosella*, Dyar), of the fall armyworm (FAW) (*Spodoptera frugiperda*, J. E. Smith), and of the European corn borer (ECB) (*O. nubilalis*, Hübner).

MATERIALS AND METHODS

^1H and ^{13}C NMR were measured at 80 and 20 MHz with tetramethylsilane (Me_4Si) and 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) as the internal standards. ^1H NMR data are the result of a simple first-order analysis and of comparison with the 6-MBOA of Venis and Watson (1978). Mass spectra were obtained by introduction of the sample into the direct inlet system of the HP 5985 mass spectrometer.

Syntheses. Syntheses were performed according to Matsumoto and Okazawa (1974). The general procedures are as follows.

Esterification. 3-, 4-, and 5-methoxysalicylic acids (Aldrich), 20 g each, were refluxed in absolute MeOH with

Table I. ^{13}C NMR Chemical Shift Values (ppm) of 4-, 5-, and 6-MBOA in $\text{Me}_2\text{SO}-d_6$

carbons	7-MBOA		5-MBOA	6-MBOA
	Me_2SO	CDCl_3		
2	154.53	155.77	155.05	154.83
4	102.79	103.10	96.46	109.76
5	124.35	124.73	156.26	109.12
6	106.73	108.12	106.85	155.25
7	143.20	143.96	109.79	97.05
8	131.76	132.63	137.52	144.14
9	131.71	130.96	131.18	123.77
CH_3O	56.20	56.90	55.74	55.77

3 g of HCl (concentrated) for 48 h. The ester was separated from the remaining acid by column chromatography on silica gel ($l = 20$ cm, $d = 5$ cm) with $\text{EtOAc}/\text{NH}_4\text{OH}$ (98/2 v/v): yield of esters, 3- = 19.1 g, 4- = 15.7 g, and 5-methyl methoxysalicylate = 17.7 g.

Hydrazide. The esters were added (without solvent) to 2.0 mol of hydrazine (Aldrich) at room temperature to form the hydrazides. Heat was evolved and the subsequent reaction mixture crystallized on standing. Excess hydrazine was removed under vacuum with a rotoevaporator: yield of hydrazides, 3- = 14.2 g, 4- = 13.9 g, and 5-substituted hydrazide = 15.0 g.

Azide. The hydrazides were dissolved with difficulty by the addition of 20 mL of concentrated HCl, 40 mL of H_2O , and then an additional 45 mL of HCl (concentrated). To a chilled solution of the hydrazides (0–5 °C), 14 g of NaNO_2 in 40 mL of H_2O , also chilled, was added slowly while maintaining the temperature of 0–5 °C over a period of 15 min. The azide was extracted from the aqueous reaction mixture with CHCl_3 : yield of azide, 3- = 8.4 g, 4- = 6.9 g, and 5-substituted azide = 10.5 g.

MBOA Synthesis. The azides, after evaporation of the CHCl_3 , were dissolved in dry toluene and refluxed overnight. The reaction product was redissolved in CHCl_3 and chromatographed on a 5 × 20 cm silica gel column, and the MBOAs were eluted with $\text{EtOAc}/\text{CHCl}_3$, 1/9 (v/v). The final MBOAs were then crystallized and recrystallized from CHCl_3 .

Physical Data. 5-MBOA: yield 8.9 g; mp 151 °C; MS m/e 165 (M^+), 150, 136, 122, 106, 94, 80, 69; ^1H NMR (CDCl_3) δ 3.80 [s, 3 H, $\text{OCH}_3\text{-C}(5)$], 6.56–6.67 [d, 1 H, H-C(6), $J = 9$ Hz], 6.68 [s, 1 H, H-C(4)], 7.04–7.16 [d, 1 H, H-C(7), $J = 9$ Hz], 9.16 (brs, 1 H, NH); ^{13}C NMR ($\text{Me}_2\text{SO}-d_6$) see Table I. 6-MBOA: yield, 4.9 g; mp 151 °C; MS m/e 165 (M^+), 150, 135, 122, 107, 97, 79, 69; ^1H NMR (CDCl_3) see Venis and Watson (1978) and Chen and Chen (1976); ^{13}C NMR (Me_2SO) see Table I. 7-MBOA: yield, 5.7 g; mp 144 °C; MS m/e 165 (M^+), 150, 122, 107,

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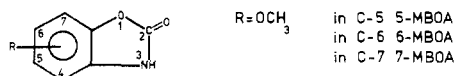


Figure 1. Structure of 5-, 6-, and 7-MBOA.

94, 79, 66; ^1H NMR (CDCl_3) δ 3.98 [s, 3 H, $\text{OCH}_3\text{-C}(7)$], 6.68–6.77 [m, 2 H, H-C(6) and H-C(5), $J_{5-6} = 8.2$ Hz, $J_{5-4} = 1.3$ Hz], 7.09–6.99 [d, 1H, H-C(4), $J = 8$ Hz], 9.31 (brs, 1 H, NH); ^{13}C NMR ($\text{Me}_2\text{SO}-d_6$) see Table I.

Bioassay Techniques. Seed growth inhibition techniques were used as previously described (Nicollier and Thompson, 1982). The bacterial bioassay with *P. maltophilia* was performed on a nutrient agar plate. The insect larval growth bioassays were performed by the methods of Davis and Hedin (1982). Newly hatched Southwestern corn borer larvae were placed on a custom Bio-Mix No. 1107 prepared by Bioserv, Inc., Frenchtown, NJ, for 5 days after which they were weighed. The diet was based on wheat germ, casein, sucrose, vitamins, salts, agar, and antimicrobial agents. The candidate compounds (0.002–0.2% of the diet) were either added directly as an aqueous solution or, if required, added in a nonaqueous solvent to the casein and rotoevaporated to remove the solvent. The casein was then mixed with the remainder of the diet. Four repetitions of five insects were employed per data point.

RESULTS AND DISCUSSION

The $^{13}\text{C}\text{-}^1\text{H}$ coupling constants, which were obtained from gated decoupling spectra with the aid of chemical shifts, led to the assignment of the resonances of the carbons in 5-, 6-, and 7-MBOA. An improved resolution was obtained upon addition of a small amount of D_2O , which gave two effects: first, the coupling to the deuteron is about one-sixth of that to the proton; second, deuteron quadrupole relaxation is much faster than proton relaxation.

Single resonance spectra may be very valuable in the case of the small-size or even medium-size molecules provided that the spectra are not too complex, but the ambiguity in the assignment of some carbons in our MBOA molecule showed that it was necessary to take account of the long-range, carbon-proton couplings.

Focusing at first on the methine carbons, C(4), C(6), and C(7) (5-MBOA), C(4), C(5), and C(7) (6-MBOA), and C(4), C(5), and C(6) (7-MBOA) (Figure 1), we found that all are doublets ($^1J_{\text{CH}}$) or doublets of doublets ($^1J_{\text{CH}}$ and $^3J_{\text{CCCH}}$). In each of the three cases, only one methine carbon exhibited no further splitting (no $^3J_{\text{CCCH}}$), which fits respectively C(7) (5-MBOA), C(4) (6-MBOA), and C(5) (7-MBOA), each having no vicinal protons. The remaining methine signals show a long-range coupling constant ($^3J_{\text{CCCH}}$) (Table II). The $^2J_{\text{CCH}}$ for those methine carbons is too small (1–2 Hz), in contrast to the vicinal coupling $^3J_{\text{CCCH}}$ (7–12 Hz) with the exception of heterocyclic aromatic compounds, where the geminal coupling can also become large according to Weigert and Roberts (1968). In our molecule, both rules can apply since the methine carbons are not in the heterocyclic aromatic ring.

The quaternary carbons C(8) and C(9) for 5-, 6-, and 7-MBOA each have different patterns depending on whether they have one or two vicinal protons. The spectrum obtained for 5-MBOA clearly reveals a symmetric seven-line pattern for the resonance at 137.52 ppm from which coupling constants can be extracted that are compatible only with C(8): $^3J_{\text{CCCH}} = 8.2$ Hz, $^3J_{\text{CCCH}} = 7.7$ Hz, and $^2J_{\text{CCH}} = 3.6$ Hz. C(9) of 5-MBOA has only one vicinal proton, and in this case, the pattern can only be a doublet of a doublet (see Table II for the value). For 6-MBOA,

Table II. Coupling Constant Values (Hertz) of 5-, 6-, and 7-MBOA

	$^1J_{\text{CH}}$	$^2J_{\text{CCH}}$	$^3J_{\text{CCCH}}$
5-MBOA	C_4H_4 , 165.3	C_5H_4 , 4.5	C_4H_6 , 5.6/4.7
		(D_2O)	
	C_6H_6 , 163.25	C_5H_6 , 4.7	C_5H_7 , 7.9
		(D_2O)	
	C_7H_7 , 166.90	C_5H_7 , 3.6	C_6H_4 , 4.9
		(D_2O)	
6-MBOA		C_9H_4 , 2.4/1.8	C_8H_6 , 7.7
		(D_2O)	(D_2O)
	C_4H_4 , 165.90	C_5H_4 , 4.5	C_6H_4 , 6.9
	C_5H_5 , 162.20	C_5H_4 , 4.1	C_7H_5 , 5.0/4.7
	C_7H_7 , 165.25		C_8H_4 , 8.7
			C_9H_5 , 9.75
7-MBOA	C_4H_4 , 167.80	C_5H_4 , 3.2	C_4H_6 , 8.0/8.2
	C_5H_5 , 163.00		C_6H_4 , 8.0/7.7
	C_6H_6 , 162.70		C_7H_5 , 8.0
			C_8H_4 , 7.9
			C_9H_5 , 9.2

Table III. Biological Evaluations of 5-, 6-, and 7-MBOA

bioassay type	compounds		
	5-MBOA	6-MBOA	7-MBOA
radish seedling ^a	10	25	10
tomato seedling ^a	10	25	10
<i>P. maltophilia</i>	0 ^b	++ ^c	
Southwestern corn borer, ED ₅₀ , %	0.20 ^d	0.40	0.40
fall armyworm, ED ₅₀ , %	>0.50	>0.50	0.40
European corn borer, ED ₅₀ , %	0.05	0.12	0.175

^a Growth in 100 ppm of test compound; percent of control. ^b Not active. ^c 100 μg applied to a bacteriological test disk paper produced in 10-mm clear zone on a nutrient agar inoculated with the test bacteria. ^d ED₅₀ = percent in diet required to reduce larval growth by 50%.

the situation is reversed for C(8) and C(9), even if C(9) does not present the complete pattern of seven lines. Carbons 8 and 9 of 7-MBOA have the same shift when the spectrum is recorded in Me_2SO solution. The pattern, therefore, after gated decoupling is quite complicated and appears as $d \times d$ with another doublet under the $d \times d$ lines, but it is still possible to determine the coupling constant (see Table II). The spectrum of 7-MBOA was also obtained in CDCl_3 to resolve C(8) and C(9).

The quaternary carbons bearing the methoxyl group C(5), C(6), and C(7) appear differently. C(5) (5-MBOA) gives a doublet of a doublet as well as C(6) (6-MBOA) even if this last one [C(6)] is hidden by C(2). Carbon 7 (7-MBOA) appears as an unresolved multiplet and is more shielded than the same carbons bearing a methoxyl group for 5- and 6-MBOA. This is due to the mesomeric effect of the oxygen of the five-membered ring. This effect is also observed for C(8) in 7-MBOA and for (8) in 5-MBOA.

The three compounds inhibited the growth of radish (*Raphanus sativus*) and tomato (*Lycopersicon esculentum*) seedlings, but only 6-MBOA was effective in reducing the growth to 15% of the control at 50 ppm (see Table III). Both 7-MBOA and 5-MBOA were inactive at 50 ppm. The inhibition of growth has been attributed by Venis and Watson (1978) to the inhibition of auxins.

Only 6-MBOA inhibited the growth of the bacterium *P. maltophilia* in an agar plate bioassay. When incorporated in laboratory diets, 5- and 6-MBOA inhibited the larval growth of three insects, the Southwestern corn borer, the fall armyworm, and the European corn borer.

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Multiple Discriminant Analysis in the Analytical Differentiation of Venetian Wines. 3. A Reelaboration with Addition of Data from Samples of 1979 Vintage Prosecco Wine

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Nineteen samples of Prosecco white wine, vintage of 1979, were analyzed for sodium, potassium, calcium, magnesium, chloride, pH, total acidity, phosphorus, ash content, and alkalinity of the ashes. Some parameters showed relevant differences with respect to the 1977 data. The new data were added to that already in our file, and the multiple discriminant analysis for the differentiation of the white wines Soave Classico, Prosecco di Conegliano-Valdobbiadene, and Verduzzo del Piave was applied. The apparent error rate obtained with all the parameters in the analysis was about 8.2%. Classification was improved (apparent error rate 6.6%) when the parameters calcium, alkalinity of the ashes, and ash content were excluded from the analysis. In this case the expected actual error rate, obtained by the jackknife procedure, was 9.8%. The number of parameters to be used can be reduced to five, without an error increment, or to four, with an apparent error rate of about 10%.

In previous papers, it was shown that the problem of differentiation of wines by means of analytical parameters can be successfully dealt with by statistical treatment of the experimental data according to the method of discriminant analysis (Moret et al., 1980; Scarponi et al., 1981). In such a method, the parameters are combined into one or more linear functions (the discriminant functions), the coefficients of which are computed to maximize the separation between the groups of different wine samples and to minimize the spread within the groups. Once the discriminant functions are obtained, it is possible to calculate values of them for each sample examined (discriminant scores) and, from these, to carry out the differentiation and the classification of the wines. The optimal combination of several parameters allows one to obtain a better exploitation of discriminant information contained in the data and to gain real means of differentiating the wines according to their origin.

Previous treatments of experimental data concerning the Venetian white wines Soave Classico, Prosecco di Conegliano-Valdobbiadene, and Verduzzo del Piave, vintage of 1977, showed that the three wines could be differentiated both in two by two comparisons, by binary discriminant analyses (Moret et al., 1980), and in a ternary comparison, by multiple discriminant analysis (Scarponi et al., 1981). The following analytical determinations were utilized as discriminating parameters: sodium, potassium, calcium,

magnesium, chloride, pH, total acidity (TA), phosphorus, ash content (AC), and alkalinity of the ashes (AA).

The values of the selected parameters can, however, change significantly from year to year, especially as a consequence of climatic and environmental factors. Then, to obtain the discriminant functions that can be valid through the years, it is necessary to introduce into the analysis the data obtained from several years, so as to weigh the parameters according to their variability through the years.

In this paper we report the results of the analytical measurements mentioned above and performed on samples of Prosecco wine, vintage of 1979. Some significant differences from the previously obtained results (vintage of 1977) are evidenced. The new data were added to that already in our file, and the multiple discriminant analysis was repeated. Discriminant and classification function coefficients are reported, and the differentiation of the wines is evaluated.

EXPERIMENTAL SECTION

Collection and Analysis of Samples. Nineteen samples of Prosecco wine, vintage of 1979, were collected in the production zone from lots for which genuineness and typicality were guaranteed and certified by the Italian D.O.C. (Denominazione di Origine Controllata—Certified Brand of Origin) brand. This brand is obtained only by producers that follow the production disciplinary rules ("Gazzetta Ufficiale della Repubblica Italiana", 1969). These rules prescribe that, in order to be classified as D.O.C., the wine, in addition to being judged by its orga-

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