Solubility of Benomyl in Water at Different pHs and Its Conversion to Methyl 2-Benzimidazolecarbamate, 3-Butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidazole, and 1-(2-Benzimidazolyl)-3-*n*-butylurea

Raj P. Singh[1] and Mikio Chiba*

The solubility of benomyl in aqueous buffers at pH 1, 3, 5, 7, 8, 9, 10, 11, 12, and 13 was measured by high-performance liquid chromatography (HPLC) at room temperature. An ultrasonic homogenizer was used to disperse the sample of benomyl in water, and then the mixture was centrifuged. Dissolved benomyl in the supernatant was converted to 3-butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidazole (STB) at pH 13 and then analyzed by HPLC. The solubility of benomyl in the pH range of 3–10 was very low, being 1.8–4.0 μg/mL. The solubility was higher at pH 1 and at pH greater than 11. At pH 13 no benomyl was found as a result of its quantitative conversion to STB. Methyl 2-benzimidazolecarbamate was found in all the solutions prepared, but 1-(2-benzimidazolyl)-3-*n*-butylurea was found only in highly alkaline solutions. Measurement of UV spectra of solutions is suggested as a convenient means to judge proximate compositions of solutes present in the solution.

The fungicide benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, has been on the market for the past 10 years, but its mode of action is not clearly understood. The primary reason for this is that benomyl is extremely difficult to dissolve in water. In addition, several other factors make the mode of action of benomyl difficult to study.

The greatest difficulty arises from the fact that benomyl decomposes in water (Clemons and Sisler, 1969; Peterson and Edgington, 1969) as well as in organic solvents (Chiba, 1977a; Chiba and Cherniak, 1978; Chiba and Doornbos, 1974). Second, its major degradation compound methyl 2-benzimidazolecarbamate (MBC) is also fungitoxic (Clemons and Sisler, 1969; Peterson and Edgington, 1969). Third, there was not any suitable analytical method to individually determine low concentrations of benomyl and MBC in water until recently (M. Chiba and R. Singh, unpublished results).

Because of the difficulty in dissolving benomyl in water, researchers have been using organic solvents to prepare high concentrations of its stock solutions, but this practice simply complicated the problem. The degradation of benomyl to MBC is dependent on time, temperature, and solvent (Chiba, 1977b). Decomposition of benomyl in water was found to be rather slow (Baude et al., 1973) or slower than that in common organic solvents (Chiba, 1975). Accordingly, a variable level of degradation occurs during the periods of sample preparation, efficacy test, and final analysis. Under these conditions, it is essentially impossible to control benomyl concentrations in test solutions. It is also impossible to carry out any benomyl experiments without having variable quantities of organic solvents and MBC in test solutions.

The fact that MBC is also fungitoxic and its level of activity is different from that of benomyl (Hall, 1980) makes the interpretation of experimental results very difficult. It is essentially impossible to judge the efficacy of chemicals when researchers do not know exact concentrations of chemicals they are using at the beginning and the end of one experiment.

Chemistry Department, Brock University, St. Catharines, Ontario L2S 3A1, Canada (R.P.S.), and Research Station, Agriculture Canada, Vineland Station, Ontario L0R 2E0, Canada (M.C.).

[1]Present address: Department of Chemistry, State University of New York, Buffalo, NY.

Calmon and Sayag (1976a) reported that benomyl rapidly decomposes to MBC at a pH lower than 5. They also confirmed the finding of White et al. (1973) that benomyl decomposes to 3-butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidezole (STB) in alkaline conditions. STB further degrades to 1-(2-benzimidazolyl)-3-*n*-butylurea (BBU) under very high pH conditions (White et al., 1973; Calmon and Sayag, 1976b). These experiments, however, were done in the presence of methanol (1:1 v/v).

To date, the only value reported as the solubility of benomyl is 3.8 ppm at 20 °C and pH 7 (Austin et al., 1976). Without having accurate solubility data at different pHs, it is rather difficult to accurately understand the behavior of benomyl in water. Extensive studies, therefore, were conducted to investigate the solubility of benomyl under different pHs in the range of 1–13. The study was further extended to identify all the degradation compounds and determine their quantities using a recently developed HPLC method (M. Chiba and R. Singh, unpublished results).

MATERIALS AND METHODS

Chemicals. *Benomyl.* Analytical standard, obtained from E. I. du Pont de Nemours and Co., Inc., and Benlate 50% WP were used.

MBC. Analytical standard was obtained from E. I. du Pont de Nemours and Co., Inc. Also, benomyl in Benlate 50% WP was heated in boiling methanol, and MBC thus formed was recrystallized (Chiba and Veres, 1980).

STB. Benomyl, recrystallized from Benlate 50% WP, was dissolved in 0.1 N NaOH solution by shaking in a separatory funnel, and the solution was filtered through a Whatman No. 1 filter paper. STB, which was formed during the above process, was precipitated by adding 1 N HCl to bring the pH down to 1.0. The precipitated STB was washed with 1 N HCl to remove BBU or MBC and then washed with distilled water. The crystal was dried in a vacuum desiccator over silica gel. Infrared and mass spectra of the crystal obtained were in good agreement with those reported by White et al. (1973), but there was a little difference in the ^1H NMR spectrum (deuterated dimethylformamide was used as a solvent); a signal for the lactam–lactim tautomer was present at 3.16 ppm. The empirical formula obtained from the mass abundance table was $C_{13}H_{14}N_4O_2$. The results of elemental analysis were C = 60.59, H = 5.33, and N = 21.66, and these agreed well with calculated values of C = 60.46, H = 5.46, and N = 21.69.

0021-8561/85/1433-0063$01.50/0

BBU. Benomyl, prepared as above, was kept in 1.0 N NaOH solution, at room temperature for 4 days. The precipitate formed was filtered, washed several times with 0.1 N NaOH solution, redissolved in 1 N HCl solution, and then reprecipitated by adjusting the solution's pH to 7 with 1 N NaOH solution. The precipitate was filtered and washed well with distilled water and dried over silica gel in a vacuum dessicator. Infrared and mass spectra of the crystal obtained were in good agreement with those of BBU reported by White et al. (1973). A difference was noticed, however, in our NMR spectra where all the three nitrogen protons plus two *N*-methylene protons resonated together at about 3.2 ppm. The results of elemental analysis confirmed the empirical formula of $C_{12}H_{16}N_4O$; calculated values were C = 62.05, H = 6.94, and N = 24.12, and found values were C = 62.19, H = 6.83, and N = 24.07.

2-Aminobenzimidazole (2-AB). Analytical standard was obtained from E. I. du Pont de Nemours Co., Inc.

Solvents. Acetonitrile and methanol used were all HPLC grade from Caledon Laboratories, Ltd., Georgetown, Ontario L7G 4R9, Canada.

Standard Solutions. Standard solutions of MBC, STB, and BBU were prepared in methanol at 100 μg/mL and then diluted to appropriate concentrations (5–10 μg/mL) with pH 7 buffer. The STB standard solutions thus prepared were used as standards for determination of benomyl after its conversion to STB.

Buffer Solutions. Buffer solutions of pH 1–13 were prepared as follows: pH 1 with 0.2 M KCl (68 mL) and 0.2 M HCl (182 mL); pH 3 with 0.1 M citric acid (159 mL) and 0.2 M Na_2HPO_4 (41 mL); pH 5 with 0.067 M Na_2HPO_4 (2.4 mL) and 0.067 M KH_2PO_4 (197.6 mL); pH 7 with 0.067 M Na_2HPO_4 (122 mL) and 0.067 M KH_2PO_4 (78 mL); pH 8 with 0.067 M Na_2HPO_4 (189 mL) and 0.067 M KH_2PO_4 (11 mL); pH 9 with 0.025 M borax (246 mL) and 0.1 M NaOH (4.0 mL); pH 10 with 0.05 M $NaHCO_3$ (206 mL) and 0.1 M NaOH (44 mL); pH 11 with 0.05 M Na_2HPO_4 (231 mL) and 0.1 M NaOH (19 mL); pH 12 with 0.05 M Na_2HPO_4 (162 mL) and 0.1 M NaOH (88 mL); pH 13 with 0.2 M KCl (69 mL) and 0.2 M NaOH (181 mL).

Instrumentation. The spectrophotometer used was Beckman Model DU-8 with a scanning system. NMR spectra were obtained with the Bruker Model WP 80 CW. Mass spectra (MS) were obtained on a AEI Model MS-30 double-beam spectrometer equipped with a Kratos DS-55 data system. The pH meter used was a Corning Scientific Instruments Model 12 research pH meter. A Biosonik ultrasonic homogenizer from Bronwill Scientific was used for initial dissolution of benomyl. The centrifuge used for the absorption spectra study was a Sorvall Super Speed Model RC2-B, equipped with an automatic refrigeration system. Another centrifuge, a Model CL International Clinic centrifuge, was used for the HPLC study.

HPLC Instruments and Operating Conditions. *HPLC.* A Perkin-Elmer Series 3 equipped with a Perkin-Elmer LC-55-S detector at 286 nm was used at room temperature of 25–27 °C.

Column. A Regis Hi-Chrom reversible column, 5-μm Spherisorb ODS (C-18) 15 cm × 4.6 mm (i.d.), was used. A precolumn [5 cm × 4.6 mm (i.d.)], dry packed with CO PELL ODS 25–37 μm (Whatman), was used along with the above analytical column.

Injector. A Rheodyne syringe loop type injector was used with 10 μL as a standard sample size.

Mobile Phase. The following two mixtures were prepared: (A) CH_3CN–H_2O–buffer (pH 7), 40:45:15 v/v; (B) CH_3CN–H_2O–buffer (pH 7), 60:30:10 v/v. Each phase was run isocratically.

Flow Rate. The flow rate was fixed at 0.8 mL/min regardless of the mobile phase.

Procedure. *HPLC Analysis.* To a 50-mg sample of Benlate (except at pH 1, 11, and 12 where 150-mg samples and pH 13 where 200-mg samples were used) 25 mL of each buffer solution was added. The ultrasonic homogenizer treatment was done with a small diameter probe at the probe intensity of 60 for 2 min. During this procedure, the sample container was kept in water to control the temperature of the sample below 25 °C. The mixture was then centrifuged for 20 min. Part of the resultant supernatant solution was taken, and pH of the solution was adjusted to 7 with the pH 7 buffer. The prepared solution was used for analysis of MBC, STB, and BBU by HPLC using mobile phase B. For accurate determination of benomyl concentration, the pH of the solution was brought to 13 to quantitatively convert beomyl to STB, then readjusted to pH 7, and analyzed by HPLC using mobiel phase A. Further details of this HPLC method are described elsewhere by Chiba and Singh. After the analysis, the excess benomyl at the bottom of the centrifuge tube was resuspended in the remaining supernatant solution and recentrifuged. The HPLC analysis of the supernatant solution was repeated after 30 min to ensure that the benomyl concentration had reached saturation. Experiments were repeated 3 times at pH 7 to ensure the reproducibility of results. Some of the samples thus prepared and standards of MBC, STB, and BBU were scanned for UV absorptions as below. For determination of solubilities of MBC, STB, and BBU, samples were treated exactly in the same manner as above.

Spectrophotometric Method. To a 10-mg sample of benomyl 100 mL of test buffer solution was added and homogenized with the ultrasonic homogenizer for 3 min by using a small diameter probe at the probe intensity of 40. During this procedure, the sample container was kept in ice-cold water to control the temperature of the sample suspension at 1 °C. The mixture was then centrifuged for 15 min at 1 °C and at 8000 rpm (*g* factor of 7720). The supernatant solution was scanned by UV absorption immediately after the centrifugation and several times thereafter. The spectrophotometer was scanned in the wavelength range of 210–310 nm with the scan speed at 50 nm/min. Standard solutions of MBC, STB, and BBU were prepared in appropriate concentrations with buffer solutions and scanned as above. By use of a 1-cm silica cell, the absorbance span was adjusted in the range of 0.1–4.0 depending on the sample concentrations.

RESULTS AND DISCUSSION

HPLC Analysis. Under the conditions given with the use of mobile phase A, all the components were clearly resolved, and retention times of STB, MBC, 2-AB, BBU, and benomyl were 3.8 4.6, 7.8, 9.0, and 26.8 min, respectively (Figure 1). The chromatogram of those compounds that are eluted with mobile phase B is shown in Figure 2. Concentrations of benomyl, which were determined as STB at different pHs, are summarized in Table I. These concentrations represent the solubilities of benomyl. The solubilities are low in the pH range from 3 to 10 but higher under strongly acidic (pH 1) and alkaline (pH 11 and 12) conditions. The increased solubility at pH 1 and pH 11 and 12 is probably due to higher populations of ionized species at these pH extremes. There was no trace of benomyl at pH 13 because it quantitatively converted to STB. The lowest solubility found was 1.8 μg/mL at pH 10.

Solubility will be influenced not only by pH but also by temperature and ionic strength of solvents. Solubility data,

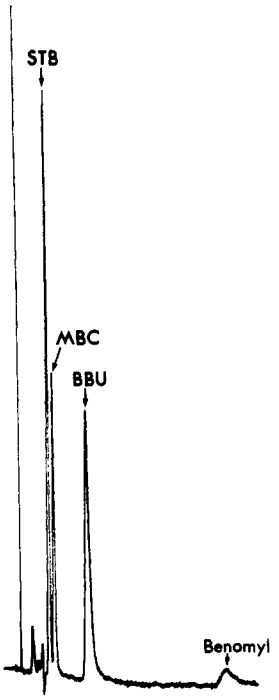

Figure 1. Chromatogram of STB, MBC, BBU, and benomyl, eluted with mobile phase A.

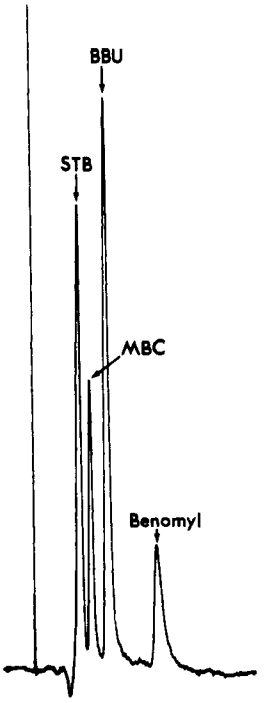

Figure 2. Chromatogram of STB, MBC, BBU, and benomyl, eluted with mobile phase B.

therefore, may be different if a similar test is conducted with different buffer solutions or with different concentrations of same buffer solution. The data given in this paper, however, will give fairly good indications as to the level of solubilities of benomyl at different pHs.

As shown in Table I, all the solutions tested contained MBC. High concentrations of MBC at lower pHs are due to the decomposition of benomyl, because MBC concentrations increased as time elasped. High concentrations of MBC at higher pHs, however, are mainly due to higher solubility of MBC. Because an excess amount of benomyl sample was used to ensure its saturation, MBC present as an impurity in the sample preferentially dissolved in water

Table I. Solubility of Benomyl and Concentrations of MBC, STB, and BBU in Buffer Solutions at Different pHs (μg/mL) When Benlate 50% WP[a] Was Used as the Source of Benomyl

	concentrations, μg/mL			
pH	benomyl[b]	MBC	STB[c]	BBU[c]
1	18.2 (24.2)[d]	57.0	ND[e]	ND
3	4.0 (54.8)	3.3	ND	ND
5	3.6 (69.2)	1.6	ND	ND
7	2.9[f] (70.7)	1.2	ND	ND
	2.8[g]	[g]	ND	ND
8	3.0 (41.7)	4.0	0.2	ND
9	1.9 (32.2)	1.1	2.9	ND
10	1.8 (14.4)	2.2	8.5	ND
11	8.8 (4.5)	24.0	164	trace[h]
12	4.5 (0.7)	57.0	547	trace
13	ND	125	2000	35

[a] This product contained 51.3% benomyl and 2.2% MBC. [b] The values indicate the solubility of benomyl. [c] Calculated as benomyl. [d] Percent of benomyl in total solutes. [e] No sign above the noise level. [f] Average of three determinations; coefficient of variation was 7.9%. [g] With pure benomyl. Because pure benomyl is so light the standard period of solubilizing procedure was not enough to saturate benomyl. It was saturated after 24 h, but by then the MBC concentration was increased to 4.0 μg/mL. [h] Less than the minimum detectable level of 0.02 μg/mL.

Table II. Absorbance of Benomyl, MBC, STB, and BBU at the λ_{max} of Each Compound[a]

compound	λ_{max}	pH	absorbance
benomyl	294	b	0.77
MBC	286	7	0.65
STB	291	7	0.52
	274	11	0.53
BBU	293	12	0.73

[a] Concentrations are all at 10 μg/mL. [b] Dissolved in acetonitrile.

and ended at higher concentrations at all pHs tested. The highest percentage of benomyl found was 70.7% at pH 7 with 29.3% of MBC in the solution.

In addition to MBC, concentrations of STB consistently increased as alkalinity increased. BBU was found only as a trace at pH 11 and 12 and a measurable quantity at pH 13; the results substantiate the previous work of Calmon and Sayag (1976b). Those concentrations of STB and BBU shown in Table I do not represent the solubility of these compounds. The concentrations of STB and BBU found in the solutions simply represent the quantities of those compounds that were converted from benomyl during the solubility test procedure; the concentrations were controlled by the rate of conversion of benomyl to STB and the rate of subsequent conversion from STB to BBU at different pHs.

The solubility of MBC was 16.0, 6.9, and 8.3 μg/mL, at pH 5, 7, and 9, respectively. Similarly, the solubilities of STB and BBU at pH 7 were 10.5 and 3.1 μg/mL, respectively.

UV Spectra of Benomyl, MBC, STB, and BBU. A typical UV spectrum of pure benomyl solution prpared in acetonitrile with the presence of butyl isocyanate (BIC) at 7 μg/mL is shown in Figure 3A. MBC and STB, both prepared in a mixed solvent of CH_3OH and pH 7 buffer (10:90 v/v), at 10 μg/mL are shown in parts B and C of Figure 3, respectively. STB prepared in a mixed solvent of CH_3OH and pH 11 buffer (10:90 v/v), and BBU, prepared in a mixed solvent of CH_3OH and pH 12 buffer (10:90 v/v), both at 10 μg/mL, are shown in parts D and E of Figure 3, respectively. Absorbance values at λ_{max} of each compound in the above solutions (10 μg/mL) are summarized in Table II. Because these spectra are widely different, the spectra of standard solutions prepared at

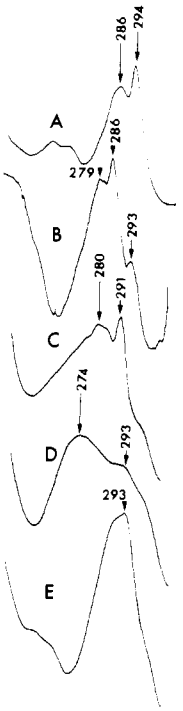

Figure 3. Absorption spectra of benomyl and related compounds. (A) Benomyl solution (7 ppm) prepared in CH_3CN with the presence of excess BIC; (B) MBC solution (10 ppm) prepared in a mixed solvent of CH_3OH and pH 7 buffer (10:90 v/v); (C) STB solution (10 ppm) prepared in a mixed solvent of CH_3OH and pH 7 buffer (10:90 v/v); (D) STB solution (10 ppm) prepared in a mixed solvent of CH_3OH and pH 11 buffer (10:90 v/v); (E) BBU solution (10 ppm) prepared in a mixed solvent of CH_3OH and pH 12 buffer (10:90 v/v).

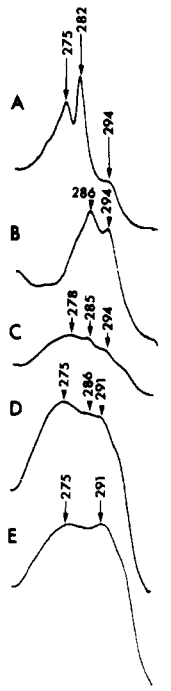

Figure 4. Typical absorption spectra of supernatant aqueous solutions of benomyl (Benlate 50% WP) at different pHs at 1 °C. (A) At pH 3; (B) at pH 7; (C) at pH 9; (D) at pH 11; (E) at pH 13.

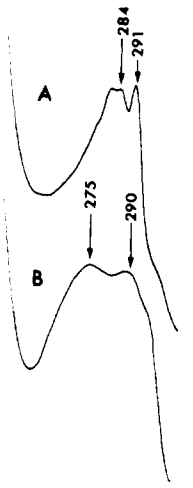

Figure 5. Typical absorption spectra of benomyl (Benlate 50% WP) buffer solutions at 25 °C; benomyl was initially dissolved in pH 12 buffer and then diluted 50 times. (A) With pH 7 buffer; (B) with pH 12 buffer.

specific conditions can be used as a reference when unknown samples in which different proportions of these compounds are found under the same conditions as those of standard solutions. Two spectra of STB obtained at pH 7 (Figure 3C) and at pH 12 (Figure 3D) are substantially different.

Spectra, obtained immediately after benomyl solutions were prepared at pH 3, 7, 9, 11, and 13, are shown in parts A, B, C, D, and E of Figure 4, respectively. The spectrum of Figure 4A is to show the presence of similar concentrations of both benomyl and MBC at pH 3 as demonstrated in Table I. At pH 7, the spectrum is very similar to that shown by Chiba (1977a) when the solution consists of benomyl and MBC with a little higher percentage of benomyl in the solution (Figure 4B). The spectra obtained at pH 5 and 8 are very similar to that at pH 7. The spectrum obtained at pH 9 (Figure 4C) is quite different from any spectra obtained at lower pHs and does not show any prominent absorption; this indicates the presence of benomyl, MBC, and STB. The spectra obtained at pH 11 and 13 (parts D and E of Figure 4) are similar to that of pure STB (Figure 3D), because STB, converted from benomyl at the defined pHs, is the major component in these solutions with the presence of other compounds as shown in Table I. All the above absorption spectra changed gradually over the period of 4–7 h; this change indicates the slow degradation of benomyl to MBC at pH 3 and 7, to MBC and STB at pH 9 and 11, and to STB initially and to BBU via STB at pH 13.

The presence of MBC in all the sample solutions is not only due to the decomposition of benomyl but also due to the presence of MBC as impurity in the benomyl sample used (M. Chiba and R. Singh, unpublished results), and due to its greater solubility than that of benomyl. Under these condtions, it is impossible to obtain MBC-free benomyl solutions in water. When MBC is present, the absorption of benomyl at 294 nm is influenced by the absorption of MBC and the reading of absorbance is dependent on the solvent used. Accordingly, it is difficult to assess the accurate concentration of benomyl in water by the UV absorbance. However, approximate concentratiosn of benomyl in water at different pHs and the presence of other compounds can be assessed conveniently by this simple technique.

Parts A and B of Figure 5 show the absorbance spectra of the mixture of benomyl, MBC, and STB when benomyl was dissolved at pH 12 and then diluted to 10 μg/mL with pH 7 and pH 12 buffer solutions, respectively. It is clear from these results that in order to obtain approximate composition and concentrations of solutes by absorbance, the pH of sample solutions should be adjusted to that of standard solutions, for example, to pH 7, because the spectra of solutes are pH dependent.

CONCLUSION

The results of this study revealed that the solubility of benomyl is low in the pH range of 3–10, being less than 4 ppm. At pH 1, its solubility is substantially higher, and at pH 13 no benomyl can be found because it completely converts to STB.

MBC was present in all the samples tested and its quantity increased as acidity and alkalinity increased. Accordingly, the percentage of benomyl in the solution, as part of other solutes, MBC, STB, and BBU, is very low. The highest value obtained under the experimental conditions tested was 70.7% at pH 7, but this value declines as benomyl converts to MBC or STB.

In practice, benomyl is being used widely for different purposes. In the citrus industry in Florida, formulated 50% WP is suspended at the pH range of 7.6–10.5 for variable periods up to 6 weeks or longer (Hall, 1980). Researchers prepare their benomyl test solutions in water with the aid of organic solvents (Koller et al. 1982; Peterson and Edgington, 1969). Under these conditions actual solute compositions will be widely different. Results obtained in this study can be utilized to help understand probable compositions of solutes when benomyl is prepared as a solution or suspension in water.

ACKNOWLEDGMENT

We thank T. Johnes for his technical assistance and R. G. Richards and L. Bigger for their assistance in measuring UV spectra.

Registry No. STB, 41136-38-3; MBC, 10605-21-7; BBU, 24374-77-4; benomyl, 17804-35-2.

LITERATURE CITED

Austin, D. J.; Lord, K. A.; Williams, I. H. *Pestic. Sci.* **1976**, *7*, 211.
Baude, F. J.; Gardiner, J. A.; Han, J. C. Y. *J. Agric. Food Chem.* **1973**, *21*, 1084.
Calmon, J. P.; Sayag, D. R. *J. Agric. Food Chem.* **1976a**, *24*, 311.
Calmon, J. P.; Sayag, D. R. *J. Agric. Food Chem.* **1976b**, *24*, 314.
Chiba, M. "Abstracts of Papers", 170th Nattonal Meeting of the American Chemical Society, Chicago IL, 1975; American Chemical Society: Washington, DC, 1975; PEST 127.
Chiba, M. *J. Agric. Food Chem.* **1977a**, *25*, 368.
Chiba, M. *Bull. Environ. Contam. Toxicol.* **1977b**, *18*, 285.
Chiba, M.; Cherniak, E. A. *J. Agric. Food Chem.* **1978**, *26*, 573.
Chiba, M.; Doornbos, F. *Bull. Environ. Contam. Toxicol.* **1974**, *11*, 273.
Chiba, M.; Veres, D. F. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1291.
Clemons, G. P.; Sisler, H. D. *Phytopathology* **1969**, *59*, 705.
Hall, D. J. *Proc. Fla. State Hortic. Soc.* **1980**, *93*, 341.
Koller, W.; Allen, C. R.; Kolattukudy, P. E. *Pestic. Biochem. Physiol.* **1982**, *18*, 15.
Peterson, C. A.; Edgington, L. V. *J. Agric. Food Chem.* **1969**, *17*, 898.
White, E. R.; Bose, E. A.; Ogawa, J. M.; Manji, B. T.; Kilgore, W. W. *J. Agric. Food Chem.* **1973**, *21*, 616.

Received for review December 16, 1983. Revised manuscript received April 26, 1984. Accepted September 18, 1984. R.P.S. was supported by a grant from the National Sciences and Engineering Research Council of Canada (NSERC).

Effects of Dietary Monensin on Bovine Fatty Acid Profiles

William N. Marmer,* Robert J. Maxwell, and Donald G. Wagner

Variations in fatty acid profiles of bovine tissue were evaluated as a function of the incorporation of the antibiotic monensin into a forage regimen. Fatty acid profiles were obtained for the separate neutral lipid and polar lipid fractions from longissimus muscle and for the total lipid content of subcutaneous adipose tissue. Statistical analysis showed no significant differences in lipid content and only few such differences in the content of most individual fatty acids when comparing control tissue to tissue from animals fed with monensin. Decreased amounts of total saturated fatty acid observed in tissue from monensin-fed animals suggested reduced biohydrogenation in the rumen, consistent with the inhibition of rumen bacterial growth. Increased amounts of odd chain length and branched fatty acids in the same tissue were consistent with increased rumen production of propionate over acetate, with subsequent de novo synthesis of the fatty acids from propionate.

The ionophore antibiotic monensin often is added to cattle feed to increase efficiency of feed utilization. Monensin alters the growth and metabolic activity of Gram-positive rumen bacteria (results of studies of protozoal activity are equivocal), causing a shift in fermentation products toward increased production of propionate and reduced production of acetate and methane (Richardson et al., 1976; Bergen and Bates, 1984). Although many nonlipid metabolic changes associated with the presence of monensin in the bovine diet have been elucidated (Richardson et al., 1976; Potter et al., 1976; Raun et al., 1976; Schelling, 1984), there have been no reports on the effects of monensin on bovine lipid composition, despite the role played by rumen microflora (Christie, 1978) on bovine lipid patterns. The present work was done to detect such effects on bovine lipid patterns, as measured by alterations in tissue content and in fatty acid composition. The experimental protocol was similar to that of a prior study, which uncovered numerous instances of significant differences in specific fatty acid content as a function of bovine dietary regimen (forage vs. grain; Marmer et al., 1984). In both studies, the examination of the fatty acids of separate neutral and polar lipid fractions

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118 (W.N.M. and R.J.M.), and Oklahoma State University, Animal Science Department, Stillwater, Oklahoma 74078 (D.G.W.).

0021-8561/85/1433-0067$01.50/0