is not possible because of agronomic factor variations (Table I). Sample consolidation needed for scaled up resin extractions precluded comparing the various cultivar 593 shipments. However, cultivar pair N576 and 12229 and cultivar pair 11634 and 11635 have cultivation and harvest histories similar enough for comparison of component distributions.

Buchanan et al. (1978) reported 7.1% polyphenolics (tannins and flavonoids) in whole plant extract. We found that polyphenolics, 37% of the aqueous extract, correspond to 5% of cultivar 593 woody tissue. The crude polysaccharide fraction, 9–10% of cultivar 593 woody tissue, is a mixture reported to contain extractable levulins, inulins, and monosaccharides (Traub and Slattery, 1946), among other non-phenolic polar components. Dilute acid hydrolysis of this fraction gave a multicomponent mixture. The most abundant monosaccharide, slowly fermented by brewer's yeast, had an HPLC retention corresponding to that of mannose. A relatively low level of arabinose was also observed. No fructose was detected. The polysaccharide fraction is not a good source of fermentable sugars.

Our program's sampling and processing protocols were directed toward woody tissue extract analysis; whole shrub extracts would require further characterization. Extract yield and composition will depend on shrub strain, cultivation history, and processing procedures. In addition, seasonal shifts in secondary metabolite distributions have been noted for guayule (Meeks et al., 1950; Lloyd, 1911). A more comprehensive extract composition profile must take this seasonal variation into account.

ACKNOWLEDGMENT

G. P. Hanson of the Los Angeles State and County Arboretum and D. D. Rubis and D. J. Garrot, Jr., of the University of Arizona provided cultivated shrub for this program. D. J. Burlett, W. D. Vandever, and R. E. Pugh were responsible for shrub processing and extraction. T. L. Folk performed mass spectral analyses of extract components.

Registry No. Guayuline A, 31685-97-9; guayuline B, 31685-98-0; argentatin A, 31324-30-8; argentatin B, 31300-41-1; argentatin C, 31300-42-2.

LITERATURE CITED

Banigan, T. F.; Verbiscar, A. J.; Weber, C. W. *J. Agric. Food Chem.* **1982**, *30*, 427.
Belmares, H.; Jimenez, L. L.; Ortega, M. *Ind. Eng. Chem. Prod. Res. Dev.* **1980**, *19*, 107.
Buchanan, R. A.; Otey, F. H.; Russell, C. R.; Cull, I. M. *J. Am. Oil Chem. Soc.* **1978**, *55*, 657.
Burlett, D. J.; Vandever, W. D.; Miller, J. W., Jr., presented at the 2nd annual Guayule Rubber Society Meeting, Phoenix, AZ, Oct 1981.
Campos-Lopez, E.; Neavez-Camacho, E.; Ponce-Velez, M. A.; Angulo-Sanchez, J. L. *CHEMTECH* **1979**, *9*, 50.
Dorado Bernal, E.; Nombela, M.; Monteil, P. *Chim. Ind. (Paris)* **1962**, *87*, 612.
Eagle, F. A. *Rubber Chem. Technol.* **1981**, *54*, 662.
Haagen-Smit, A. J.; Fong, C. T. O. *J. Am. Chem. Soc.* **1948**, *70*, 2075.
Haagen-Smit, A. J.; Siu, R. *J. Am. Chem. Soc.* **1944**, *66*, 2068.
Hendrickson, J. B.; Rees, R. *Chem. Ind. (London)* **1962**, 1424.
Keller, R. W.; Winkler, D. S.; Stephens, H. L. *Rubber Chem. Technol.* **1981**, *54*, 115.
Klein, G.; Pirschle, K. *Biochem. Z.* **1923**, *143*, 457.
Lloyd, F. E. "Guayule, a Rubber-Plant of the Chihuahuan Desert"; Carnegie Institute: Washington, DC, 1911; pp 183–187.
Mears, J. A. *J. Nat. Prod.* **1980**, *43*, 708.
Meeks, J. W.; Banigan, T. F. U.S. Patent 2744125, 1956.
Meeks, J. W.; Banigan, T. F., Jr.; Planck, R. W. *India Rubber World* **1950**, *12*, 301.
Proksch, P.; Behl, H. M.; Rodriguez, E. *J. Chromatogr.* **1981**, *213*, 345.
Rodriguez, E.; Reynolds, G. W.; Thompson, J. A. *Science (Washington, D.C.)* **1981**, *211*, 1444.
Rodriguez-Hahn, L.; Romo de Vivar, A.; Ortega, A.; Aguilar, M.; Romo, J. *Rev. Latinoam. Quim.* **1970**, *1*, 24.
Romo, J.; Romo de Vivar, A.; Ortega, A.; Diaz, E. *Rev. Latinoam. Quim.* **1970**, *1*, 132.
Schmid, L.; Stoehr, R. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1408.
Swett, C. E. *J. Ind. Eng. Chem.* **1909**, *1*, 315.
Traub, H. P.; Slattery, M. C. *Bot. Gaz. (Chicago)* **1946**, *108*, 295.
Walter, E. D. *J. Am. Chem. Soc.* **1944**, *66*, 419.

Received for review November 17, 1982. Accepted February 28, 1983. This work was supported in part by a grant (DA 1073) from the U.S. Department of Commerce, administered by the Institute of Polymer Science of the University of Akron. This work was presented at the 3rd annual Guayule Rubber Society Meeting, El Paso, TX, Aug 24–26, 1982. Contribution No. 643 from The Goodyear Tire & Rubber Company.

Influence of pH and Light on the Kinetics of Vitamin B_6 Degradation

Bouchta Saidi and Joseph J. Warthesen*

A method based on ioning pairing high-performance liquid chromatorgraphy was used to measure the stability of pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM). Kinetic analysis was used to compare the influence of various factors on the rate constants. In buffered solution in the pH range of 4–7, PN showed very little degradation while PM and PL showed a strong influence of pH on loss rates. In a dry model system exposed to different temperatures, water activities, and light, loss of PL was significantly influenced by storage temperature and light.

The instability of vitamin B_6 during processing and storage can contribute to losses of the nutritional quality of foods. This vitamin can occur as pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM, and the phosphorylated forms of these compounds. Since the various forms do not have the same stability (Hassinen et al., 1954; Gregory and Kirk, 1978a; Ang, 1979), an evaluation of the loss reactions must take into account the reactivity of each vitamer.

While B_6 is recognized as an unstable vitamin (Harris and Karmas, 1975), few studies have taken a kinetic approach to evaluate the factors that influence the stability of vitamin B_6. Gregory and Kirk (1978a) demonstrated that the degradation of various B_6 vitamers in a model

University of Minnesota, Department of Food Science and Nutrition, St. Paul, Minnesota 55108.

0021-8561/83/1431-0876$01.50/0 © 1983 American Chemical Society

system at 37 °C could be described by first-order kinetics. Rate constants were also calculated by Evans et al. (1981) for the degradation of PN at elevated temperatures. Navankasattusas and Lund (1982) applied kinetic analysis to the thermal destruction of B_6 vitamers in buffer solution at pH 7.20 and in cauliflower puree.

In addition to the effects of thermal processing and possibly Maillard browning (Gregory and Kirk, 1978a), B_6 vitamers are known to be light sensitive, especially at higher pH levels (Cunningham and Snell, 1945; Reiber, 1972; Ang, 1979). Light-induced degradation could be of practical significance in some foods exposed to light and yet little information has been reported on the effects of light under specific conditions. The role of pH in B_6 vitamer stability is unclear since, for the pH range of most foods, some suggest there is no pH effect (Harris and Karmas, 1975). However, at extremes in pH (Cunningham and Snell, 1945) or at high pH in conjunction with light (Ang, 1979), pH seems to affect vitamer stability. In either case, the effects of light or pH on B_6 vitamer stability have not been studied by using a kinetics approach.

Because foodstuffs have varied distributions of the vitamers (Toepfer and Lehmann, 1961; Gregory et al., 1981) and the stability of the vitamers differs, it is useful to study the degradation as individual compounds. Several methods based on high-performance liquid chromatography (HPLC) have been suggested for use in vitamin B_6 analysis (Williams et al., 1973; Wong, 1978; Gregory and Kirk, 1978a; Vanderslice et al., 1979; Morita and Mizuno, 1980; Gregory et al., 1981). The selection of an HPLC method depends on the vitamers being analyzed, the presence of other compounds, and other chromatographic requirements of analysis.

The objective of this research was to use a kinetics approach to evaluate the role of pH on vitamin B_6 stability and to evaluate the influence of heat and light on the degradation of B_6 vitamers.

EXPERIMENTAL SECTION

Chromatography of B_6 Vitamers. A Model M-45 solvent delivery system (Waters Associates) was used in conjunction with a Model 7010 Rheodyne fixed loop (50 μL) injector. Separations were performed on a C_{18} μBondapak column (3.9 mm × 30 cm, Waters Associates). The detector was a Farrand Ratio Fluorometer 2 with a 10-μL quartz flow cell. The excitation filter was a 295-nm narrow band interference filter and the emission was through a 4-70 filter (cutoff below 340 nm). Detector output was recorded on a Hewlett-Packard 3380A recorder–integrator.

Two different mobile phases were used for the HPLC analysis of the three B_6 vitamers. The first mobile phase was 0.033 M sodium phosphate buffer (pH 2.2) pumped at a flow rate of 2.0 mL/min (Gregory and Kirk, 1978a). A second mobile phase was developed in this study which provided more chromatographic flexibility. The solvent contained an ion pairing agent and an organic modifier to control elution time. The composition was 88% 0.03 M sodium phosphate buffer (pH 3.0) containing 0.002 M sodium octanesulfonate (Regis Chemical Co.) and 12% acetonitrile. When pumped at 1.0 mL/min, PN, PL, and PM eluted in less than 10 min.

For quantitation, standard solutions of the hydrochloride salts of PN, PL, and PM (Sigma Chemical Co., stated purity approximately 98%) were prepared in 0.03 M sodium phosphate buffer (pH 2.5) and were stored at 4 °C in low actinic glassware. All samples and standards were handled under conditions of subdued light. Peak height was used for quantitation of the standards and the samples. A standard curve was prepared for calibration and also to show that peak height response was linear with concentration.

Sample Treatments. To determine the effect of pH on the three nonphosphorylated vitamers of B_6, separate solutions of the hydrochloride salts of PN, PL, and PM at a concentration of 2.0 μg/mL were prepared at pH 4.0, 5.0, 6.0, and 7.0 by using a citrate–phosphate buffer system as reported by Gomori (1955). Approximately 5 mL of each treatment was placed in a 10-mL glass test tube with a Telfon-lined screw cap. The tubes were held in covered water baths at either 40 or 60 °C for up to 140 days. Duplicate tubes were sampled periodically and analyzed by HPLC for the B_6 vitamers.

To test the effects of temperature and light on B_6 vitamers, a fortified dry model system similar to that described by Gregory and Kirk (1978b) was used. The model system had the following composition: 50% cornstarch, 30% sucrose, 14% microcrystalline cellulose, 5% casein, and 1% sodium chloride. The system was fortified with either PN·HCl, PL·HCl or PM·2HCL to a level of 70 μg/g. The systems were blended with distilled water into a slurry and then frozen at −10 °C. The samples were then freeze-dried, mixed, and placed in Petri dishes 5 cm in diameter to a depth of about 1 mm. The samples were then equilibrated in the dark at 21 °C over saturated salt solutions. Desiccators with saturated $MgCl_2$ were used for a water activity (a_w) of 0.32, and saturated K_2CO_3 was used to achieve a a_w of 0.44. Equilibration was determined by weighing until a constant weight was obtained. This required 5 days.

The stability of the vitamers in the model system was examined at temperatures of 5, 28, 37, and 55 °C, at a_w's of 0.32 and 0.44, and at light intensities of 200 and 400 ft-c (2150 and 4300 lm/m^2, respectively). To maintain constant conditions, the samples were held in custom-built chambers designed to control a_w and light intensity (Woodcock et al., 1982). Light was controlled by variable intensity standard fluorescent bulbs (Cool White, General Electric No. F15T8-CW) mounted on the top of the chamber. The chambers were placed in controlled-temperature rooms, and duplicate samples were removed periodically for extraction and analysis by HPLC.

The B_6 vitamers were extracted from the dry model system by using an acidic buffer solution. The procedure was modified from that of Gregory and Kirk (1978a) to use 0.03 M sodium phosphate buffer (pH 2.5) in place of acetate buffer. A 1.0-g sample was mixed with 25 mL of the buffer for 2 min on a Vortex mixer and then centrifuged at 2000g for 15 min. After filtration through a 0.45-μm membrane filter, the supernatant was used directly for HPLC analysis. Recovery from the model system was determined by adding 1 mL of a 30 μg/mL solution of each vitamer to 1.0 g of the unfortified model system. Recoveries ranged from 77 to 100%. For the stability studies, the loss of each vitamer was calculated as a difference from an identical but untreated control sample. Percent recovery was assumed to be the same for the control and the treatment so no recovery correction was used in the data analysis.

Data Treatment. Linear regression was used to determine if the degradation of the vitamers followed first-order kinetics. Rate constants, k, were determined, and analysis of variance was used to determine if the effects of various treatments were significant.

RESULTS AND DISCUSSION

The analysis of B_6 vitamers by HPLC provided a convenient approach to collecting quantitative data for kinetic

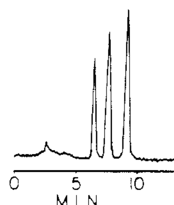

Figure 1. Chromatogram of vitamin B_6 standards using a mobile phase of 88% 0.03 M phosphate buffer (pH 3.0) containing 0.002 M sodium octanesulfonate and 12% acetonitrile. Elution order is PL, PN, and PM.

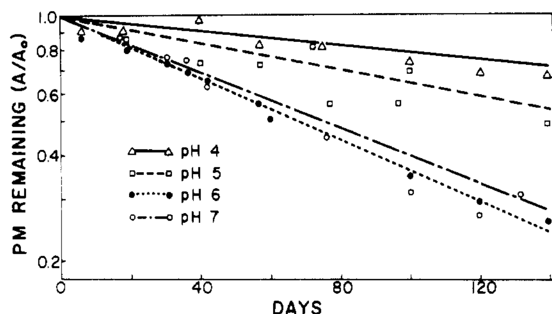

Figure 2. Retention of PM in solutions held at 60 °C.

analysis. In initial work, the mobile phase was 100% phosphate buffer which gave elution of the vitamers in less than 5 min. However, the mobile-phase composition could not be adjusted to increase the elution time of the B_6 vitamers. In some samples this became a problem because other compounds eluted at about the same time as the vitamers, giving peaks with shoulders and making quantitation difficult. Variations were also noted in the retention time of the vitamers when sample composition was changed.

An alternative to standard reverse-phase chromatography for the separation of the three nonphosphorylated vitamers is ion pair chromatography (Williams, 1979; Morita and Mizuno, 1980). When numerous combinations of acetonitrile or methanol and heptane- or octanesulfonic acid in phosphate buffer were used, an optimum mobile phase providing resolution of the vitamers was developed. Figure 1 is a chromatogram showing the elution of the B_6 vitamers with a mobile phase of 12% acetonitrile and octanesulfonic acid as the counter ion. The order of elution is PL, PN, and PM with detection limits of 6, 4, and 3 ng, respectively. These limits of detection are about the same as with the mobile phase of 100% phosphate buffer. Detector response for each vitamer was linear in the range of concentrations tested (0.1–2.0 μg/mL).

The ion pairing mobile phase that was developed in this study provides some chromatographic flexibility because the amount of acetonitrile can be varied when changes in elution time are desired. Another advantage is that two completely different mobile phases (the phosphate buffer and ion pairing system) can be used to help verify the identity of B_6 vitamers in unknown samples. Use of octanesulfonic acid for ion pairing with the B_6 vitamers appears to introduce sufficient lipophilic character to allow a later, more controllable elution of the polar vitamers.

The influence of pH on the rate constants of vitamin B_6 degradation was determined in buffered solutions rather than foods or dry systems so that pH control could be more precise. When solutions of PN at pH levels from 4 to 7 were held at 40 and 60 °C for up to 140 days, no significant degradation was observed. This precluded any kinetic analysis of degradation or any influence that pH might exert on vitamer stability. PN is generally considered the

Table I. Effect of pH and Temperature on Rate Constants and Half-Life for PM Degradation in Solution

T, °C	pH	k, days^{-1}	correlation coeff (r^2)	half-life, days
40	4.0	0.0017	0.90	467
	5.0	0.0024	0.90	289
	6.0	0.0063	0.98	110
	7.0	0.0042	0.96	165
60	4.0	0.0021	0.62	330
	5.0	0.0044	0.94	157
	6.0	0.0110	0.99	63
	7.0	0.0108	0.98	64

Table II. Effect of pH and Temperature on Rate Constants and Half-Life for PL Degradation in Solution

T, °C	pH	k, days^{-1}	correlation coeff (r^2)	half-life, days
40	4.0	0.0002	0.46	3466
	5.0	0.0017	0.93	407
	6.0	0.0011	0.95	630
	7.0	0.0009	0.80	770
60	4.0	0.0011	0.73	630
	5.0	0.0225	0.97	31
	6.0	0.0047	0.63	147
	7.0	0.0044	0.86	157

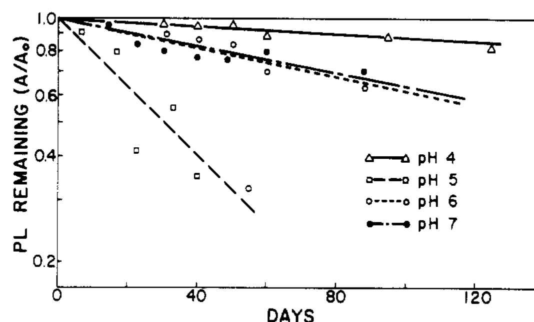

Figure 3. Retention of PL in solutions held at 60 °C.

most stable B_6 form and the solution stability results would support that conclusion.

Under the same conditions, PM showed a significant effect of temperature and pH. Figure 2 illustrates the loss of PM at 60 °C, and Table I shows the calculated rate constant, correlation coefficient for a first-order rate plot, and half-life for each treatment. Poorest correlation coefficients were obtained at pH 4 and 5, probably because the amount of loss was small and low levels of degradation are not conducive to determining reaction order (Benson, 1960). Conditions causing greater losses of the PM have a high correlation coefficient, suggesting that the degradation is proceeding by first-order kinetics. Gregory and Kirk (1978a) have previously indicated that B_6 vitamer loss followed first-order kinetics in dehydrated food systems. Navankasattusas and Lund (1982), using 5 mM solutions of the vitamers in buffered solution, showed that thermal processing of B_6 vitamers could be modeled by pseudo-first-order, 1.5-order, and second-order rate constants.

When rate constants were compared for the 40 °C treatments, pH 4 and pH 5 were found to have the same rate constants at the $P = 0.05$ level. These were lower than pH 6 and 7, which had similar rate constants at the $P = 0.05$ level. At 60 °C, the rate constants were significantly different from each other except between pH 6 and pH 7. PM degradation shows a trend of increasing rate with increased pH. For example, at 60 °C and pH 6, the PM half-life was 63 days compared to a value of 330 days at pH 4. The temperature effect was also significant ($P =$

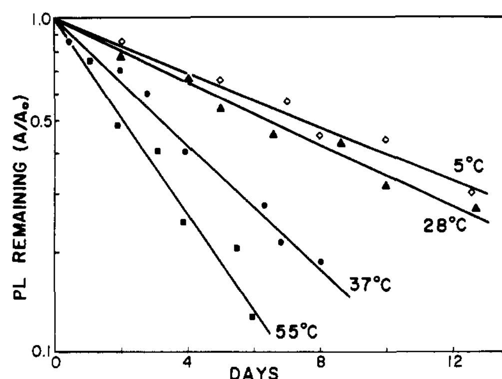

Figure 4. Retention of PL in a model system of $0.32a_w$ and 400 ft-c.

0.05) at each pH except 4 where losses were very small.

PL was relatively stable throughout the incubation period. Figure 3 shows the loss of PL at 60 °C. Table II presents the rate constant, correlation coefficient, and half-life for each treatment. At both temperatures, only pH 6 and pH 7 were found to have similar rate constants at $P = 0.05$; pH 4 had the lowest, and pH 5 had the highest rate constants. This is in contrast to PM, which showed an increase in loss with increasing pH up to pH 6–7. Except for pH 4, the two temperatures showed a significant difference ($P = 0.05$) in rate constant with the 60 °C treatments having the higher values at each pH.

In samples of PM and PL where degradation was extensive, an apparent degradation peak appeared in the chromatogram during vitamer analysis. Using the two different mobile phases described under Experimental Section and a standard solution of 4-pyridoxic acid, the peak was tentatively identified as 4-pyridoxic acid, based on the retention time. The amount of this compound was small, so that further qualitation or quantitative analysis in these samples was precluded. 4-Pyridoxic acid is an expected degradative product of the B_6 vitamers (Snell, 1963; Reiber, 1972; Gregory and Kirk, 1978a), but further experiments would be required to better characterize the relationship between vitamer loss and 4-pyridoxic acid formation.

It should be noted that transamination reactions are possible with the B_6 vitamers in food systems but were unlikely in the buffered systems used in this experiment.

The information gathered on the effect of pH on vitamin B_6 stability indicates why the influence of pH on B_6 degradation in foods is unclear. Under identical conditions, PN showed no loss, PM showed greater losses at high pH, and PL showed greatest loss at pH 5 and less loss above and below that pH. Demonstrating the mechanisms by which pH influences vitamer stability is beyond the scope of this study. The reasons for the varied pH effects on the three vitamers may lie in the rather complex ionic equilibria that exist for the various B_6 forms (Snell, 1963).

A dry model system was used to determine the influence of light on vitamer stability because the opaque nature of the powder made it more similar to a food system than would have been possible with a clear liquid solution of the vitamers in a glass container. When dry model systems containing either PN or PM were exposed to 400 ft-c of light at temperatures ranging from 5 to 55 °C, only small amounts of degradation occurred. After 15 days, losses ranged from 8 to 22%, but these decreases were not large enough or uniform enough for kinetic analysis. When the model system contained PL, losses were substantial as shown in Figure 4 for samples equilibrated to a water activity of 0.32. Results for samples held at 0.44 water activity were similar to those at 0.32. The correlation coefficients shown in Table III suggest that the degradation of PL in this system also follows apparent first-order kinetics. The rate constants at 5, 28, and 55 °C at each a_w were compared to determine the influence of a_w in PL losses. The rate constants for the two a_w's were not different at the 0.05 level. This may be because the water activities studied were in a narrow range and a wider range of water activities would have to be used before concluding that water activity had, or did not have, an effect on PL stability in this system. However, it appears that water activity in the range of 0.32–0.44 is not a major factor in the light-induced degradation of PL in a low-moisture system.

Table III. Effect of Temperature, Water Activity, and Light Intensity on Rate Constants and Half-Life for PL Degradation in a Model System

light intensity, ft-c	a_w	T, °C	k, days^{-1}	correlation coeff (r^2)	half-life, days
400	0.32	5	0.092	0.98	7.4
		28	0.1085	0.95	6.4
		37	0.2144	0.88	3.2
		55	0.3284	0.91	2.1
	0.44	5	0.0880	0.98	7.9
		28	0.1044	0.93	6.6
		55	0.3453	0.93	2.0
200	0.32	27	0.0675	0.90	10.3

A statistical comparison of the rate constants within each water activity showed that each temperature was different at the 0.05 level and an increasing temperature increased the rate of degradation. The rate constants for PL loss at $a_w = 0.32$ and 27–28 °C showed a significant difference for samples exposed to 200 and 400 ft-c. When the light intensity doubled, the rate constant increased by a factor of 1.5. The half-life at 200 ft-c was 10.3 days compared to 6.4 days at 400 ft-c.

When the activation energy was calculated from the slope of an Arrhenius plot for each model system, the value for $a_w = 0.32$ was 4.8 kcal/mol (correlation coefficient of 0.85) and for $a_w = 0.44$, 5.0 kcal/mol (correlation coefficient of 0.84). This again, shows very little effect of a_w on the reaction. The somewhat low correlation coefficients might be a result of studying a light-induced degradation reaction in a dehydrated system where light penetration may not be unform in all samples because of slight variations in sample depth. While the activation energy for light-induced degradation of PL would be affected by light intensity, light penetration, pH, and other factors, the values are much lower than those reported for thermal destruction of PL (Navankasattusas and Lund, 1982). Activation energies for light-induced degradation have not been previosly reported for the B_6 vitamers, but the values are within the range found in studies on the light-induced degradation of riboflavin. Although direct comparisons are not possible because of differences in light intensity and food systems, Woodcock et al. (1982) reported activation energies for riboflavin degradation in macaroni in the range of 0.6–4.3 kcal/mol, while Singh et al. (1975) reported 8.0 kcal/mol for milk exposed to light in glass containers.

The results from the dry model system study indicate that PL is much more light sensitive than PN or PM. When compared to another light-sensitive vitamin such as riboflavin (Woodcock et al., 1982), the rate constant for PL degradation would appear to be lower than the rate constant for riboflavin degradation under similar conditions by a factor of 10–20. However, further work appears

necessary to more completely characterize the light stability of B_6 vitamers in food systems under various conditions.

Registry No. PN, 65-23-6; PL, 66-72-8; PM, 85-87-0.

LITERATURE CITED

Ang, C. Y. W. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 1170.
Benson, S. W. "Foundations of Chemical Kinetics"; McGraw-Hill: New York, 1960.
Cunningham, E.; Snell, E. E. *J. Biol. Chem.* **1945**, *158*, 491.
Evans, S. R.; Gregory, J. F., III; Kirk, J. R. *J. Food Sci.* **1981**, *46*, 555.
Gomori, G. *Method Enzymol.* **1955**, *1*, 138.
Gregory, J. F.; Kirk, J. R. *J. Food Sci.* **1978a**, *43*, 1801.
Gregory, J. F.; Kirk, K. R. *J. Food Sci.* **1978b**, *43*, 1585.
Gregory, J. F.; Manley, D. B.; Kirk, J. R. *J. Agric. Food Chem.* **1981**, *29*, 921.
Harris, R. S.; Karmas, E. "Nutritional Evaluation of Food Processing", 2nd ed.; AVI Publishing Co.: Westport, CT, 1975.
Hassinen, J. B.; Durbin, G. T.; Bernhart, F. W. *J. Nutr.* **1954**, *53*, 249.
Morita, E.; Mizuno, N. *J. Chromatogr.* **1980**, *202*, 134.
Navankasattusas, S.; Lund, D. B. *J. Food Sci.* **1982**, *47*, 1512.
Reiber, H. *Biochim. Biophys. Acta* **1972**, *279*, 310.
Singh, R. P.; Heldman, D. R.; Kirk, J. R. *J. Food Sci.* **1975**, *40*, 164.
Snell, E. E. In "Comprehensive Biochemistry"; Elsevier: New York, 1963; Vol. 11, Chapter 4.
Toepfer, E. W.; Lehmann, J. *J. Assoc. Off. Anal. Chem.* **1961**, *44*, 426.
Vanderslice, J. T.; Stewart, K. K.; Yarmas, M. M. *J. Chromatogr.* **1979**, *176*, 280.
Williams, A. K. *Methods Enzymol.* **1979**, *62*, 415.
Williams, R. C.; Baker, D. R.; Schmit, J. A. *J. Chromatogr. Sci.* **1973**, *11*, 618.
Wong, F. F. *J. Agric. Food Chem.* **1978**, *26*, 1444.
Woodcock, E. A.; Warthesen, J. J.; Labuza, T. P. *J. Food Sci.* **1982**, *47*, 545.

Received for review December 14, 1982. Accepted April 4, 1983. This project was supported in part by the University of Minnesota Agricultural Experiment Station, Project No. 18-87, and University of Minnesota IAV Hassan II Contract No. AID-PRO-AG-608. Paper No. 13054, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

Determination of Penicillin G, Penicillin V, and Cloxacillin in Milk by Reversed-Phase High-Performance Liquid Chromatography

William A. Moats

A method is described for determination of three commonly used monobasic penicillins, penicillin G, penicillin V, and cloxacillin, in milk by high-performance liquid chromatography. Penicillins were extracted from milk with 2:1 acetonitrile. Cleanup was accomplished by partitioning between buffers and organic solvents, first at acid (pH 2.2) and then at neutral (pH 7.0) pHs. For HPLC, a reversed-phase C_{18} column was used. The solvent system was (A) 0.01 M H_3PO_4 and (B) acetonitrile. A solvent gradient of 80 A:20 B–40 A:60 B in 20 min, flow rate 1 mL/min, was used to elute all penicillins studied within 20 min. Recoveries of penicillins from spiked samples were 88–105%. Limits of detection were about 0.002 ppm for cloxacillin and 0.005 ppm for penicillin G and V. The procedure should be satisfactory for most monobasic penicillins but did not work with the more polar carbenicillin and amphoteric compounds such as ampicillin.

Methods have been described for determination of penicillins in pharmaceutical preparations by high-performance liquid chromatography (HPLC) using both reversed-phase (LaBelle et al., 1979; Larsen and Bundgaard, 1978; Tsuji et al., 1979; White et al., 1975) and anion-exchange columns (Blaha et al., 1975; Tsuji and Robertson, 1975) and also in blood serum using reversed-phase HPLC (Thijssen, 1980; Vree et al., 1978).

Thijssen (1980) and Vree et al. (1978) used perchloric acid to precipitate proteins in the determination of respectively isoxazoyl penicillins and ampicillin and amoxicillin in blood serum by HPLC. Although precipitation of proteins with strong acid appears satisfactory for acid-stable penicillins, it is unsuitable for some penicillins which are rapidly degraded under acid conditions (Hou and Poole, 1971).

Meat Science Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD 20705.

The application of HPLC methods to determination of penicillin residues in milk and animal tissue has not previously been reported. Detection of penicillin at the residue levels required by regulatory agencies requires isolation from more complex substrates and much higher sensitivity than is required for clinical applications. This paper describes a method for determination in milk of three penicillins commonly used in veterinary practice.

MATERIALS AND METHODS

Chemicals. Acetonitrile was UV grade and residue analysis grade, petroleum ether (30–60 °C) and methylene chloride were residue analysis grades, and other chemicals used were reagent grades. Penicillins were obtained from Sigma Chemical Co.

Equipment. A Buchler Rotary Evapomix and vortex mixer were used. All glassware was cleaned in special detergents designed for critical cleaning and rinsed in 1% hydrochloric acid and distilled water before use.

HPLC Apparatus. A Varian Model 5000 liquid chromatograph was used with a Varian UV-50 detector set at

This article not subject to U.S. Copyright. Published 1983 by the American Chemical Society