mations. Although bovine serum albumin has been used as a model protein in this study, the approach and the results presented here may help in the study of the structure–function relationship of more complex seed proteins.

ACKNOWLEDGMENT

This research was supported in part by the College of Agricultural and Life Sciences and through a cooperative agreement with the U.S. Department of Agriculture.

LITERATURE CITED

Brown, M. R. *Proc. FEBS Meet.* **1977**, *50*, 1–20.
Creighton, T. E. *J. Mol. Biol.* **1977**, *113*, 329–41.
Damodaran, S. *Anal. Biochem.* **1985**, *145*, 200–4.
Damodaran, S. *Int. J. Pept. Protein Res.* **1986**, *27*, 589–96.
Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979a**, *75*, 403–14.
Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979b**, *75*, 415–26.
Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979c**, *75*, 727–39.
Graines, G. L., Jr. *Insoluble Monolayers at Liquid–Gas Interfaces*; Interscience: New York, 1966; pp 45.
Johanson, K. O.; Wetlaufer, D. B.; Reed, R. G.; Peters, T., Jr. *J. Biol. Chem.* **1981**, *256*, 445–50.
Kinsella, J. E. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *7*, 219–80.
Kinsella, J. E.; Damodaran, S. In *Criteria of Food Acceptance*; Solms, J., Hall, R. L., Eds.; Forster: Zurich, 1980; pp 296–332.
MacRitchie, F. *Adv. Protein Chem.* **1978**, *32*, 283–324.
MacRitchie, F.; Alexander, A. E. *J. Colloid Sci.* **1963a**, *18*, 458–63.
MacRitchie, F.; Alexander, A. E. *J. Colloid Sci.* **1963b**, *18*, 453–57.
Peters, T., Jr. In *Plasma Proteins* 2nd ed.; Putnum, F. W., Ed.; Academic: New York, 1975; Vol. 1, pp 133–181.
Ter-Minassian-Saraga, L. *J. Colloid Interface Sci.* **1981**, *80*, 393–401.
Thannhauser, T. W.; Konishi, Y.; Scheraga, H. A. *Anal. Biochem.* **1984**, *138*, 181–88.
Tornberg, E. *J. Sci. Food Agric.* **1978a**, *29*, 762–76.
Tornberg, E. *J. Colloid Interface Sci.* **1978b**, *64*, 391–402.
Trurnit, H. A. *J. Colloid Sci.* **1960**, *15*, 1–13.
Ward, A. F. H.; Tordai, L. *J. Chem. Phys.* **1946**, *14*, 453–461.

Received for review June 2, 1986. Accepted October 16, 1986.

A Gas–Liquid Chromatographic Method for Analysis of Pyruvic Acid and Lower Molecular Weight Fatty Acids in Plant Materials

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A gas–liquid chromatography (GLC) method has been developed for quantitative analysis of pyruvic acid and lower molecular weight fatty acids in plant materials. Plant extracts containing pyruvic acid and other low molecular weight fatty acids were treated with sodium borohydride, converting the pyruvic acid to lactic acid. Heating in butanol saturated with dry hydrogen chloride subsequently converted the acid components into the corresponding butyl esters, which were analyzed by GLC. GLC–mass spectrometry was used to confirm the identity of the GLC peaks. The efficiency of recovery of added pyruvic acid from Coastal Bermuda grass silage was 94%. Analyses were carried out on onion, apple, banana, and wheat kernels.

Since the elucidation of the mechanism of protein synthesis in plants is of major importance, considerable attention has been devoted to this topic (Bryan, 1976). Kretovich and Kasprek (1962) demonstrated the biosynthesis of proteins from pyruvic acid in rice and sunflower seeds. Therefore, because of the role played by pyruvic acid in protein synthesis and various biochemical cycles, interest in a method of quantitating pyruvic acid in biological materials has been investigated extensively. Quantitation of α-keto acids (pyruvate) in biological materials has always presented a serious challenge because of difficulties in their isolation, separation, and identification. Keto acids were found to be difficult to extract and esterify because of their instability. Luke et al. (1963) and other investigators experienced decomposition on chromatography (Ackerman et al., 1960; Rumsey et al., 1964). Several publications have appeared dealing with differing derivatization techniques for GLC analyses of this type of compound. Silylation of α-keto acids with hexamethyldisilazane and trimethylchlorosilane in pyridine reportedly resulted in multiple products as revealed by GLC analyses (Horii et al., 1965). According to this study Me_3Si–oxime derivatives of keto acids were stable on GLC analyses and gave single peaks. We were unable to reproduce their procedure for pyruvic acid; however, this could be due to minute quantities of water present in our samples. Presence of water in the reaction mixture would interfere with formation of both oxime and the subsequent Me_3Si derivative (Pierce, 1977). Other researchers have reported GLC analysis of pyruvic acid as its methyl ester; however, they reported that two peaks were produced (Rumsey et al., 1964).

Several recent publications have reported the separation of Krebs cycle acids, dairy product acids, and acids formed by metabolism of anaerobic microorganisms on various growth media by high-precision liquid chromatography (HPLC) (Guerrant et al., 1982; Marsill et al., 1981). However, Guerrant et al. have shown that oxalacetic acid decomposes to form pyruvic acid in either acidic or basic solutions at ambient temperature. Thus, accurate quantitative analysis for pyruvic acid and oxalacetic acids in an acid fraction would require very rapid isolation of the sample followed by HPLC analyses. Even though these investigators maintain that oxalacetic acid alone decomposes in acidic or basic solution, it is well-known [cf. Merck Index (1983)] that pyruvic acid also polymerizes and decomposes on standing if impurities are present or aerobic conditions exist in storage.

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Table I. Recovery of Pyruvate as Lactic Acid

expt		% rec[a]
1	sodium pyruvate + sodium borohydride 2 h at ambient temperature + acetic acid to decompose the excess reducing agent	82
2	sodium pyruvate + sodium borohydride heated 2 h at 37 °C + acetic acid to decompose the excess reducing agent	94
3	sodium pyruvate + sodium borohydride 2 h at ambient temperature + hydrochloric acid to decompose the excess reducing agent	84
4	sodium pyruvate + sodium borohydride 2 h at 37 °C + hydrochloric acid to decompose the excess reducing agent	95

[a] Average of triplicates for each experiment.

This paper presents a method for extraction of pyruvic acid and additional lower molecular weight fatty acids from biological materials followed by reduction of pyruvate to lactic acid. The resultant lactic acid is stable and readily convertible to the corresponding butyl ester, as are the additional acids for analysis by GLC.

MATERIALS AND METHODS

Plant Materials. The acid fraction was isolated from coastal Bermuda grass silage by the following method. Triplicate 10-g samples of wet material were extracted with 100 mL of 0.06 N aqueous sodium hydroxide in a closed-glass container for 2 h with a wrist-type shaker. Pyruvate, if present in coastal Bermuda grass silage, exists below the levels of detection of our technique. Recovery of pyruvate was determined by adding known quantities (100 mg, 99%+ purity) of sodium pyruvate (Sigma Chemical Co., St. Louis, MO) to 10-g samples of wet silage. Sodium borohydride (100 mg) was added to each of the samples before shaking. Reduction was performed as follows: after 2 h of extraction and reduction as previously described, the container was opened and excess sodium borohydride was decomposed by adding concentrated hydrochloric acid dropwise with stirring until hydrogen bubbling ceased. After the observed hydrogen evolution ceased, the pH was adjusted to 8–10 by addition of 6 N aqueous sodium hydroxide. An aliquot (5 mL) was placed in a Teflon-capped test tube, frozen with dry ice, and subsequently freeze-dried. The freeze-dried sodium salts in the tubes were then converted into the corresponding butyl esters by butanol saturated with dry hydrogen chloride (0.2 mL of butanol–hydrogen chloride + 2.0 mL of chloroform). The tubes were heated for 2 h at 80 °C, cooled with tap water, and opened; 0.2 mL of trifluoracetic anhydride was added to each and the resultant mixtures then allowed to stand for 1 h at ambient temperature. The butyl ester fraction was washed with water, dried over anhydrous sodium sulfate, and subjected to GLC analysis. Standard curves of butyl lactate were prepared by plotting integrator counts vs. micrograms of pure butyl lactate over a range of 0–20 μg.

Pyruvic acid was extracted from 20-g samples of several plant materials such as onion, apple, banana, and wheat kernels by chopping with a high-speed Virtis homogenizer (45 000 rpm) for 3 min in 0.06 N aqueous sodium hydroxide. The container was cooled by an ice bath during the chopping procedure. Reduction of the pyruvate was accomplished as previously described. Samples were run in duplicate. The results are reported in Table II.

GLC–MS Analysis. Analyses were performed on a Perkin-Elmer 900 gas–liquid chromatograph equipped with a flame ionization detector on samples of 1-μL volume. The chromatograph was interfaced through an effluent splitter to a Du Pont 21-490 mass spectrometer equipped with differential pumping on the analyzer section. Separations were made on 50 m × 0.05 cm open tubular glass column coated with OV-1. GLC conditions: carrier inlet pressure, 1.1 kg/cm^2; injector and manifold temperature, 250 °C; column temperature, maintained at 60 °C for 5 min and then programmed at 2 °C/min to 225 °C. Mass spectrometer conditions: ion source temperature, 200 °C; scan rate, 10 s/decade; ionizing voltage, 70 eV; ion source pressure, 2×10^{-5} torr. The presence of butyl lactate was confirmed by comparison of the observed spectrum and GLC retention time with those of an authentic sample (Pfaltz & Bauer, Inc. Waterbury, CT). The purity of the latter standard of butyl lactate was assayed by GLC–MS. Additional GLC analyses were performed on the same column but with the following conditions: oven temperature, 50 °C for 3 min and then programmed at 8 °C/min to 270 °C; injector temperature, maintained at 230 °C; manifold temperature, 270 °C; helium inlet pressure, 1.1 kg/cm^2. A Perkin-Elmer 300 gas chromatograph with subambient temperature control was employed for these analyses. These conditions were found superior for reproducibility and resolution of the lower molecular weight acids encountered in the fractions. The amount of pyruvic acid present in the samples was determined by the following calculation: (total lactic acid) − (initial lactic acid in the sample) = yield of pyruvic acid.

Table II. Pyruvic Acid Content of Some Plant Materials

plant matl extr	pyruvic content, μmol/g
commercial red onion	6.4[a]
Golden Delicious apple	7.1
commercial red apple	20.5
banana	7.5
oasis wheat	3.7
Florida 301 wheat	4.8

[a] Results are given in micromoles per gram of wet plant material and are the mean of duplicate analyses.

RESULTS AND DISCUSSION

Varying conditions for reduction of sodium pyruvate with sodium borohydride and subsequent decomposition of excess borohydride were tried as shown in Table I. As evident from the results of 2 and 4 in the table, the optimum recovery of reduced pyruvate as lactate was obtained when the temperature was held at 37 °C when compared with those performed at ambient temperature. No significant difference in percentage recovery of reduced pyruvate was observed when hydrochloric acid rather than acetic acid was used to decompose the excess reducing agent under the same temperature conditions. Since acetic acid is commonly encountered in various biological materials, it was decided to decompose the excess borohydride with an inorganic acid, to eliminate interference with the measurement of this acid. Recovery of reduced pyruvate and subsequent butylation of the resultant lactic acid were determined by GLC analyses, and a standard curve was obtained by plotting known weights of butyl lactate vs. integrator counts from GLC peaks. Recovery of sodium pyruvate using samples of known weight and purity from wet silage material was 95%, a value comparable to those observed for recovery of reduced pyruvate in a nonbiological system (Table I). Presence of butyl lactate was confirmed by comparison of GLC retention times and mass spectra. In order to establish the precision of our technique (extraction and reduction of pyruvate from biological materials), we chose to analyze onions, which were reported to contain pyruvic acid based on a procedure involving derivitization and colorimetric analysis (Schwimmer and Weston, 1961). This study identified pyruvate (nonenzymatically produced) as being present in a number of com-

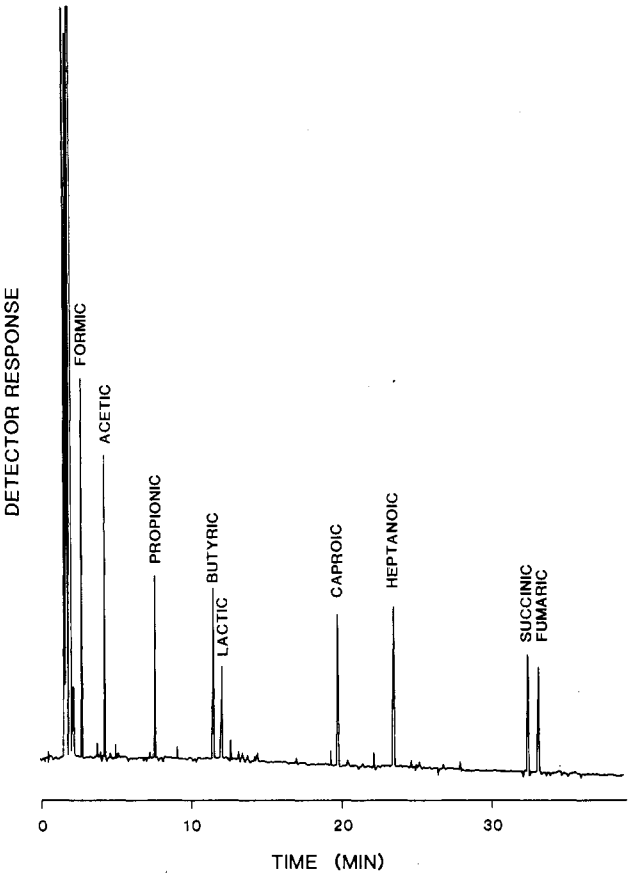

Figure 1. Gas chromatogram of the silage acids as the corresponding butyl esters.

mercial onion cultivars. The levels were determined as 2.0–4.1 micromoles per gram of fresh material. In another study, Bajaj et al. (1980) reported higher values (6.18–13.27 μmol of pyruvic acid/g fresh weight) for additional varieties of onions. Our technique yielded a value intermediate to these reported values (6.1 μmol/g fresh weight) for a red onion obtained from a commercial source (Table II). In addition, pyruvic acid in apple, banana, and wheat kernels was determined by the analytical procedure herein described. Table II lists the amounts present in these different types of plant materials. The substitution of butyl esters for the commonly used methyl esters of low molecular weight acid fractions resulted in superior resolution on capillary GLC analyses. Also, the use of a gas chromatograph equipped with subambient temperature control for these analyses produced additional resolution and better reproducibility. Figure 1 shows a typical analysis of the silage acids as their corresponding butyl esters. Note the excellent separation of acetic to butyric acids, as well as lactic acid.

The advantages of the procedure herein reported for the analysis of pyruvic acid in biological materials include simplicity, reproducibility of reactions, stability of the derivatives, and applicability for both qualitative and quantitative analyses.

Registry No. $MeCOCO_2H$, 127-17-3.

LITERATURE CITED

Ackerman, R. G.; Bannerman, M. A.; Vandenheuvel, F. A. *Anal. Chem.* **1962**, *32*, 1209.

Bajaj, K. L.; Kaur, G.; Singh, J.; Gill, P. S. *Plant Foods Hum. Nutr.* **1980**, *30*, 117.

Bryan, J. K. In *Plant Biochemistry*, 3rd ed.; Academic: New York, 1976; Chapter 17.

Guerrant, G. O.; Lambert, M. A.; Moss, C. W. *J. Clin. Microbiol.* **1982**, *16*, 355.

Horii, C.; Makita, M.; Tamura, Y. *Chem. Ind. (London)* **1965**, *34*, 1494.

Kretovich, V. L.; Kasprek, V. *Soviet Plant Physiol. (Engl. Transl.)* **1962**, *8*, 583.

Luke, H. H.; Freeman, J. E.; Kier, L. B. *Anal. Chem.* **1963**, *35*, 1916.

Marsili, R. T.; Ostapenko, H.; Simmons, R.; Green, D. E. *J. Food Sci.* **1981**, *46*, 52.

Pierce, A. E. In *Pierce Handbook and General Catalog*; Pierce Chemical Co.: Rockford, IL, 1977.

Rumsey, T. S.; Noller, C. H.; Burns, J. C.; Kalk, D.; Rhykerd, C. L.; Hill, D. L. *J. Dairy Sci.* **1964**, *47*, 1418.

Schwimmer, S.; Weston, W. J. *J. Agric. Food Chem.* **1961**, *9*, 301.

Windholz, M.; Budavari, S.; Blumetti, R. F.; Otterbein, E. S. In *Merck Index*, 10th ed.; Academic: New York, 1976, Chapter 17.

Received for review May 9, 1986. Revised manuscript received September 22, 1986. Accepted November 15, 1986. Reference to brand or firm names does not constitute endorsement by the USDA over others of a similar nature not mentioned.