Chemical and Physical Properties of Sugarcane Bagasse Irradiated with γ Rays

Youn W. Han,* Edwin A. Catalano, and Alex Ciegler

Sugarcane bagasse was irradiated with γ rays of ^{60}Co at the dose range of 0–300 Mrd, and the changes in chemical and physical properties of the bagasse were determined. Bagasse irradiated with more than 10 Mrd lost its fiber strength and became pulpy. The hemicellulose component of bagasse decomposed more readily than the cellulose and lignin fraction. Irradiation increased acid and enzyme digestibility of sugarcane bagasse. The amount of reducing sugar produced by enzymatic hydrolysis of samples irradiated with 100 Mrd was about 3 times greater than that from untreated bagasse. At or above 50 Mrd, crystallinity of sugarcane bagasse decreased and in vitro rumen digestibility increased.

Sugarcane bagasse is the fibrous residue remaining after sugarcane stalk has been crushed and the juice removed. The annual production of bagasse in the world exceeds 100 million tons, more than half of which is produced in the western hemisphere (Srinivasan & Han, 1969). Because of its fibrous nature, sugarcane bagasse has been most widely used as a fuel, paper and pulp, structural materials, and agricultural uses. Recently, due to increasing worldwide shortage of food and energy sources, sugarcane bagasse has been considered as a substrate for single cell protein (SCP) (Enriquez, 1981; Sidhu and Sandhu, 1980; Han et al., 1971), animal feed (Barhate et al., 1981; Martin and Elias, 1978), and renewable energy production (Lipinsky, 1979).

One of the major obstacles in utilizatin of the vast amount of lignocellulosic material is its recalcitrancy for digestion. Because of lignin–cellulose complex, crystallinity of cellulose, and other unknown factors the lignocellulosic plant biomass is difficult to digest biologically or chemically. Upon hydrolysis, plant biomass would yield a variety of fermentable sugars and chemicals useful for production of energy and food. A number of physical, chemical, and biological treatments have been tried to improve the digestibility of plant materials (Detroy et al., 1980; Dunlap & Chiang, 1981; Fan et al., 1980; Han et al., 1977; Johnson et al., 1976; Kleinert, 1976; Millett et al., 1976; Tassainari & Macy, 1977; Tsao et al., 1978), but because of economic constraints few have been exploited commercially. In the past, high-energy radiation also have been used to increase the digestibility of lignocellulosics (Lawton et al., 1951; Saeman et al., 1952; Charlesby, 1955; Pritchard et al., 1962), but the efforts have been considered mainly to be of academic interest. However, increasing number of nuclear power plants and the problem of disposing of large quantities of nuclear wastes induced renewed interest in using radiation in utilization of plant biomass (Bonoto and Milic, 1969; Kunz et al., 1972; Kumakura and Kaetsu, 1979; Duchacek and Bludovsky, 1979; Ibrahim and Pearce, 1980; Beardmore et al., 1980; Han et al., 1981).

In this study, we have examined the changes in chemical and physical properties of sugarcane bagasse subjected to γ irradiation and investigated the possible advantages of γ irradiation in utilization of biomass. γ irradiation extensively degraded and solubilized sugarcane bagasse in water, but direct production of glucose from biomass by γ irradiation was difficult due to further decomposition of glucose. Hydrolysis of the solubilized cellooligomer by enzyme or acid and utilization of irradiated biomass for animal feed appeared promising.

U.S. Department of Agriculture, Southern Regional Research Center, New Orleans, Louisiana 70179.

MATERIALS AND METHODS

Substrate. Sun-dried sugarcane bagasse (*Saccharum Officianarum*) was ground to pass a 1.25-cm screen, and the fiber and pith were separated by a vibratory screen. Hemicellulose-free material was obtained by treating bagasse with 1 N NaOH or 1 N H_2SO_4 for 1 h at 121 °C. These conditions were chosen based on previous experiments where most of hemicellulose in sugarcane bagasse was removed by these conditions. These samples were washed with water until the effluent became neutral. Hemicellulose was further fractionated into hemicellulose A and hemicellulose B. The former was the portion that was solubilized by 1 N NaOH and precipitated by neutralization, and the latter was the portion that was solubilized by 1 N NaOH and remained soluble after neutralization. Hemicellulose B was precipitated by addition of an equal volume of 95% ethanol. A schematic presentation of the hemicellulose fractionation is shown in Figure 1.

Hydrolysis of Sugarcane Bagasse. For acid hydrolysis, a 0.25-g sample was heated in 25 mL of 2 N H_2SO_4 at 121 °C for 2 h. For enzymatic hydrolysis, a 0.25-g sample and 50 mg of cellulase (Onozuka R-10, Yakult Biochemical Co., Japan) were mixed in 50 mL of 0.1 M acetate buffer (pH 5.0) and incubated at 50 °C with occasional shaking. A portion of reaction mixture was withdrawn at preset time intervals and filtered, and the amount of reducing sugar was determined.

Analytical Procedures. The amount of reducing sugar was analyzed by the dinitrosalicylic acid (DNS) method (Petterson and Porath, 1966) while glucose was measured by the glucose oxidase/peroxidase system (Sigma, 1978). Cell-soluble matter, hemicellulose, cellulose, lignin, ash, and in vitro rumen digestibility (IVRD) were determined according to the methods of Goering and Van Soest (1970). The crystallinity of cellulose was measured by means of an X-ray diffraction analyzer (General Electric XRD-5 diffractometer equipped with a Cu-tube, Ni-filter, proportional counter). Samples were placed in the holder and mounted in the goniometer, and the diffraction intensity was measured from an angle of $2\theta = 6–30°$. The crystallinity index was calculated as $CrI = 100(I_{22.6°} - I_{18.0°})/I_{22.6°}$, according to the method of Segal et al. (1959). Fiber strength was measured by the Instron Precision Material Testing Instrument (Instron Engineering Corp., Canton, MA), according to their instruction manual (Application series). Samples were placed in an open container (5.1-cm diameter × 2.2-cm depth), and a 1.3-cm diameter probe was forced to penetrate at 0.1 lb of force. The degree of compression (inches), recovery of the probe from compression after release of the pressure (percent), and energy to compress (inch-pounds) were determined.

Table I. Chemical Composition and Sugar Yield of Sugarcane Bagasse and Its Fractionated Components (Percent Dry Matter)

constituent	whole sugarcane bagasse	fiber	pith	NaOH-extracted bagasse	H_2SO_4-extracted bagasse
cell-soluble matter	4.2	0.9	10.1	29.6	21.8
hemicellulose	28.5	30.8	31.8	8.8	0
cellulose	50.4	53.1	39.3	56.1	63.6
lignin	14.9	13.3	15.3	4.9	22.5
ash	2.0	1.8	3.2	0.4	0
crude protein	1.59	1.31	1.85	0.23	1.01
glucose yield by acid hydrolysis	4.4	4.4	5.0	6.0	4.0
glucose yield by enzymatic hydrolysis	6.4	6.8	8.0	44.0	9.6

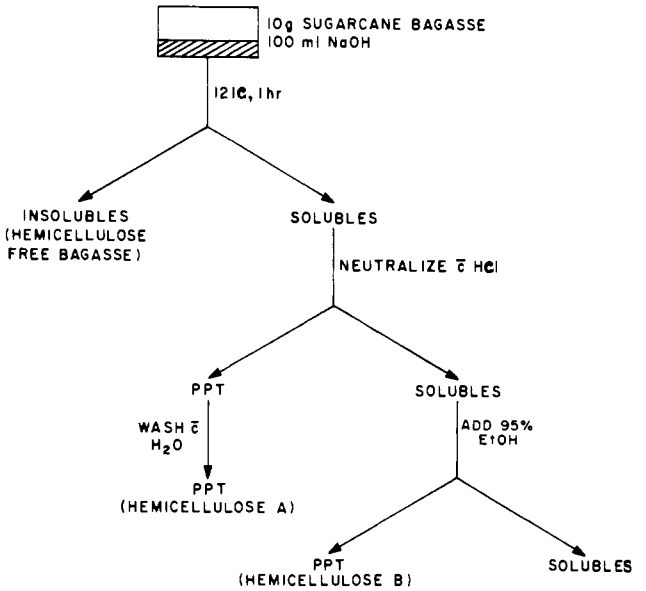

Figure 1. Isolation scheme for hemicellulose from sugarcane bagasse.

Sugars in the hemicellulose were converted into their alditol–acetate derivatives by a modified procedures of Albersheim et al. (1967). To 10.0 mL of hemicellulose hydrolysate 1.5 g of $BaCl_2 \cdot 2H_2O$ was added. The precipitate was removed by centrifugation and the clarified sample was adjusted to pH 6.0 by the addition of $Ba(OH)_2 \cdot 8H_2O$. Exactly 2.5 mL of the fluid was transferred into a 250-mL round-botton flask, and 1.0 mL of a *myo*-inositol solution containing 10 mg of the compound was added as an internal standard. The sugars in the liquid were then reduced to their respective alcohols by addition of 0.15 g of $NaBH_4$. After 30 min at room temperature, the excess $NaBH_4$ was decomposed by the dropwise addition of CH_3COOH until the evolution of gas stopped. The mixture was then evaporated to dryness on a rotary evaporator in vacuo. The borate produced from the borohydride was removed by adding 10 mL of CH_3OH and evaporating to dryness. After two more cycles of methanol addition and evaporation to dryness, the mixture was held in an oven at 100–110 °C for 10 min. Ten milliliters of $(CH_3CO)_2O$ and 0.5 g of $C_2H_3NaO_2$ were added, and the mixture was refluxed in an oil bath at 140 °C for 20 min. After the content was evaporated to dryness on a rotary evaporator, about 15 mL of CH_2Cl_2 was added to dissolve the alditol–acetate derivatives, and the mixture was then centrifuged at 11000*g* for 30 min. The supernatant liquid was decanted into a test tube and evaporated to 1 mL by gently bubbling a continuous stream of nitrogen gas through the sample. Two microliter portions of sugar samples were then analyzed with a Tracor MT 200 series gas chromatograph equipped with a flame ionization detector and a stainless steel column (180 cm × 3 mm) packed with GP 3% SP 2340 on 100/120 Supelcoport. The temperature was programmed between 150 and 250 °C at 4 °C/min.

Irradiation. Sugarcane bagasse was placed in an airtight plastic or glass container and subjected to γ irradiation at dosages of 0–300 Mrd. Irradiation was performed in a water-shielded ^{60}Co γ cell that produced a dosage of approximately 2500 rd/min. Details of the experimental apparatus are described by Calleton et al. (1970). Because the radiation source capsule was placed in a swimming pool and the samples were irradiated under water, no extra safety precaution was needed for radiation leakage. Also, there was no special problem in handling the irradiated materials as the energy level of γ source (1.33 MeV) is far less than the energy required for activation of material (5–10 MeV).

RESULTS AND DISCUSSION

Fresh sugarcane bagasse from the mills contained about 50% moisture and the dry matter contained about 50% cellulose, 28% hemicellulose, and 15% lignin (Table I). The fiber portion of the bagasse contained more cellulose whereas the pith portion contained more cell-soluble matter and lignin. On acid and enzymatic hydrolysis, the pith produced slightly more glucose than the fiber. Treatment with 1 N NaOH and 1 N H_2SO_4 removed most of the hemicellulose from the bagasse. The treated residue increased digestibility and produced more sugar than untreated bagasse. This effect was especially pronounced when bagasse was treated with 1 N NaOH, by which about 7 times more glucose was produced compared to the untreated bagasse upon enzymatic hydrolysis. The NaOH effect in increased level of sugar production, however, was not apparent on acid hydrolysis. The NaOH treatment is thus useful in overall utilization of sugarcane bagasse because it separates hemicellulose from plant biomass and the residual material improves digestibility. The NaOH-treated residue could be used for glucose production by enzymatic hydrolysis or as ruminant feed.

The beneficial effect of γ irradiation in lignocellulosic utilization have been noted by many researchers (Aoki et al., 1977; Brenner et al., 1979; Kunz et al., 1972; David et al., 1980; Ibrahim and Pearce, 1980; Kumakura and Kaetsu, 1979; Duchacek and Bludovsky, 1979; Han et al., 1981). In general, γ irradiation of lignocellulosics such as wood, paper, and crop residues causes depolymerization of biopolymers and decomposition of carbohydrates at dosages between 1 and 100 Mrd, and the resulting material shows a loss of crystallinity and an increase in digestibility by subsequent hydrolysis by acid and enzymes.

When sugarcane bagasse was irradiated with γ rays from ^{60}Co, the main effects noted were decomposition of sugarcane fiber. The radiation effects on sugarcane bagasse became apparent at 50 Mrd or greater. The hemicellulose content at this dosage decreased and cell-soluble matter increased (Table II). It was apparent that the increase in cell-soluble matter resulted primarily from solubilization

Table II. Chemical Composition of Irradiated Sugarcane Bagasse (Percent Dry Matter)

composition	radiation, Mrd					
	0	1	10	50	100	300
cell-soluble matter	4.2	12.4	12.8	24.2	48.9	93.7
hemicellulose	28.5	21.2	22.6	10.0	6.1	0
cellulose	50.4	46.2	46.0	47.5	31.9	6.1
lignin	14.9	18.1	15.9	16.0	10.7	2.1
ash	2.0	2.0	2.3	2.1	2.1	1.8

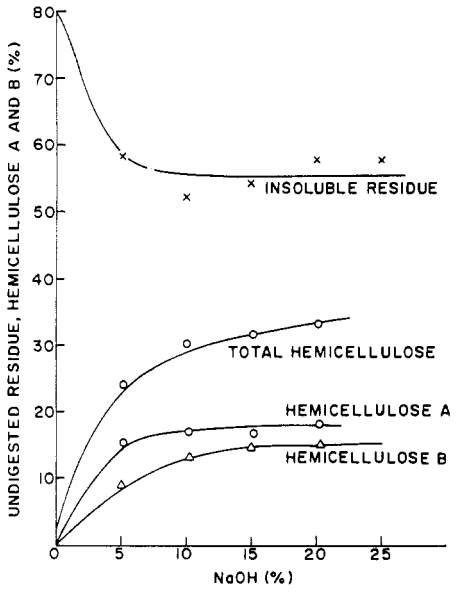

Figure 2. Hemicellulose extraction from sugarcane bagasse by different concentrations of NaOH. Extraction procedure is shown in Figure 1.

of hemicallulose; cellulose, lignin, and ash contents were little affected by a dose of 50 Mrd or less. Cellulose and lignin were decomposed at 100 Mrd or above, and most of the bagasse became water soluble at 300 Mrd or above. Contrary to the general belief (Arthur et al., 1960; Saeman et al., 1952) that the natural polymers in plant biomass cleave randomly and the crystalline and the amorphous regions of cellulose are equally affected by radiation, radiolysis of hemicellulose occurred more readily than that of cellulose or lignin. Pretreatment with swelling agents increased the solubilization of sugarcane bagasse. The sample treated with 17% NaOH and irradiated with a dose of 50 Mrd or greater completely lost the physical integrity of the fiber and formed a dark brownish paste or liquid depending on the amount of liquid in the sample.

Since hydrolysis of hemicellulose produced little glucose, removal of hemicellulose from the plant biomass prior to its hydrolysis would improve the percent glucose yield. Hemicellulose in sugarcane bagasse was easily extracted by heating the bagasse in 1 N H_2SO_4 or 1 N NaOH at 121 °C for 1 h or at room temperature for 24 h. The amount of hemicellulose extracted was increased gradually as the concentration of NaOH increased up to 10%; with higher concentration of NaOH the amounts extracted reached a plateau (Figure 2). The rate of decrease in residual bagasse was inversely proportional to that of hemicellulose removal. The hemicellulose was further fractionated into hemicellulose A and hemicellulose B. Sugarcane bagasse contained 55% hemicellulose A and 45% hemicellulose B. Chromatographic analysis revealed that the major component in hemicellulose A was xylose (88.5%), arabinose (9.1%), and galactose (2.4%), whereas hemicellulose B contained xylose (61.2%) galactose (27.8%), and arabinose (11.0%). Considerable amount of reducing sugar was

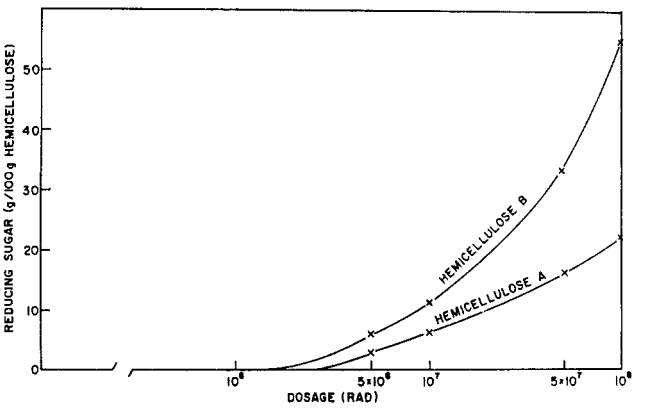

Figure 3. Sugar production from irradiated hemicellulose. Hemicellulose A and hemicellulose B (10% in aqueous solution) were irradiated at different doses, and the level of reducing sugar was determined by the DNS method.

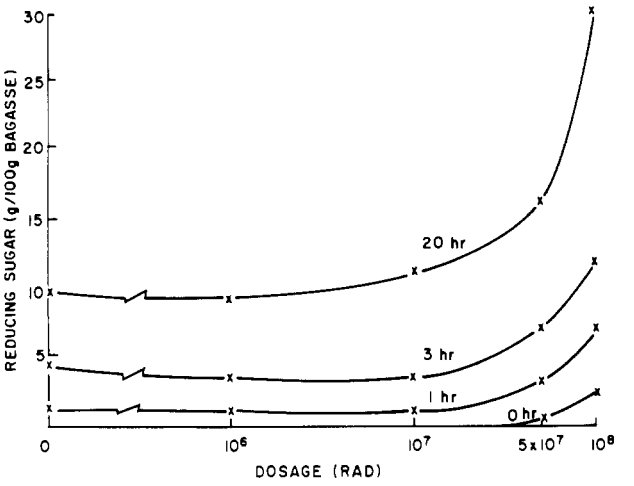

Figure 4. Effect of γ irradiation on enzymatic hydrolysis of sugarcane bagasse. The irradiated sample (0.25-g sample in 50 mL of 0.1 M acetate buffer, pH 4.5) was hydrolyzed with 50 mg of Onozuka cellulase at 50 °C.

produced upon irradiation of hemicelluloses (Figure 3). The hemicellulose B fraction was more labile than hemicellulose A fraction for radiolysis. Upon irradiation of 10^8 rd, hemicellulose B produced more than 50% (g/100 g of hemicellulose) reducing sugar whereas hemicellulose A produced about 20% reducing sugar.

The effect of γ irradiation on enzymatic hydrolysis of sugarcane bagasse is shown in Figure 4 The reducing sugar yield was higher with increasing radiation dose, and the effect became apparent when the dose level was about 10 Mrd or greater. The amount of reducing sugar produced from the sample irradiated with 100 Mrd was about 3 times greater than that produced from the unirradiated control. Kumakura & Kaetsu (1979) reported that irradiation doses of more than 100 Mrd were required to give a noticeable effect on sugar production from rice straw by enzymatic hydrolysis. On acid hydrolysis, more glucose was produced from hemicellulose-free sugarcane bagasse than whole sugarcane bagasse (Han et al., 1981). This effect was not observed in the enzymatic hydrolysis of hemicellulose-free bagasse; levels of glucose produced were about the same for whole sugarcane bagasse and hemicellulose-free bagasse obtained by either NaOH or H_2SO_4 treatment.

Radiation also caused an apparent decrease in crystallinity and increased the digestibility of lignocellulosic materials (Table III). For example, the crystallinity index of untreated sugarcane bagasse was 66.6% whereas that

Table III. Crystallinity and in Vitro Rumen Digestibility of Irradiated Sugarcane Bagasse

treatment	crystallinity, %	IVRD, %
untreated		
0 rd	66.6	38.3
1 Mrd	67.2	37.8
10 Mrd	69.0	34.8
50 Mrd	45.4	43.2
100 Mrd	44.4	50.1
acid extracted		
0 rd	73.3	
1 Mrd	70.1	24.3
10 Mrd	73.3	24.2
50 Mrd	70.0	31.2
100 Mrd	58.0	39.9
NaOH extracted		
0 rd	73.3	38.6
1 Mrd	68.7	39.3
10 Mrd	73.3	48.7
50 Mrd	72.6	43.2
100 Mrd	66.6	55.3

of bagasse irradiation with 100 Mrd was 44.4%. The effect of irradiation on crystallinity was apparent when the dosage level was more than 10 Mrd. The effect was more apparent on untreated whole bagasse than on hemicellulose-free bagasse. The in vitro rumen digestibility (IVRD) of sugarcane bagasse increased from 38.3% for untreated bagasse to 50.1% for the 100-Mrd irradiated sample. The increase in IVRD was somewhat related to the decreased in the crystallinity index.

The effect of γ irradiation on cellulose crystallinity is controversial. Some investigators (Arthur et al., 1960; Beardmore et al., 1980; Takamuku et al., 1961; Murayama, 1963) have reported that γ irradiation had no effect on cellulose crystallinity while others (Seifert, 1964; Ishiguro et al., 1973; Aoki et al., 1977; Goto et al., 1974) reported a significant change in cellulose crystallinity by γ irradiation. Arthur et al. (1960) reported that the X-ray diffraction pattern and infrared absorption curves of irradiated cotton samples retained all of the characteristics given by purified cotton without any lessening of intensity whereas the sample subjected to vibratory ball milling lost its crystallinity as indicated by a broadening and smoothing out of the characteristic peaks. The γ-irradiated sample did not show this behavior, suggesting that irradiation did not produce a decrease in cellulose crystallinity. Controversial aspects probably resulted from the crudeness of the methods used for determining crystallinity and the difference in radiation doses applied. There is no doubt that with higher dosages, e.g., over 500 Mrd, crystallinity would certainly change because the cellulose irradiated at that dosage level becomes water soluble.

γ irradiation also caused a considerable change in the fiber strength of sugarcane bagasse. As the irradiation dose increased the fiber lost its strength and became pulpy. When a probe was forced to penetrate the irradiated sample, the degree of compression, recovery of the penetrated probe, and energy required for compression were closely related to the level of dosage applied and to the appearance of physical deterioration of the material (Table IV). The sample treated with 17% NaOH and irradiated with 50 Mrd had about twice as much compressibility and half as much recovery and it took only half as much energy to compress.

Table IV. Fiber Strength of Sugarcane Bagasse Treated with NaOH and Irradiated with γ Radiation

treatment	compression,[a] in.	recovery,[a] %	energy to compress,[a] in.-lb
untreated	0.22 ± 0.025	21.9 ± 1.20	0.925 ± 0.069
10 Mrd	0.31 ± 0.011	16.6 ± 0.33	0.656 ± 0.009
17% NaOH, 10 Mrd	0.41 ± 0.046	12.4 ± 0.40	0.468 ± 0.043
17% NaOH, 50 Mrd	0.46 ± 0.040	12.4 ± 1.21	0.463 ± 0.057

[a] Values are means of four replicates with standard deviation.

CONCLUSION

γ irradiation at doses of 50 Mrd or greater extensively degraded and solubilized sugarcane bagasse in water, but direct production of glucose from the biomass by γ irradiation was not obtained because of further glucose decomposition. Hydrolysis of solubilized cellooligomers or mildly (for example, less than 10 Mrd) irraiated bagasse by cellulase or acid would be more reasonable for production of fermentable sugars. Utilization of irradiated biomass for animal feed appeared promising.

As a pretreatment method, γ irradiation is somewhat equivalent in terms of increasing the digestibility to that of NaOH treatment, one of the most commonly used method in upgrading foliage digestibility. As a hydrolysis method, however, γ irradiation has some advantages and disadvantages over the conventional acid and enzymatic hydrolysis. The simplicity of the process and the effectiveness of chain cleavage are the advantages, but loss of glucose due to indiscriminatory destruction of the product is the disadvantage. However, direct comparision of the effect of γ irradiation to that of other methods is difficult due to its different nature of causative agents and the different result it produces.

ACKNOWLEDGMENT

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Registry No. Hemicellulose, 9034-32-6; cellulose, 9004-34-6; lignin, 9005-53-2; hemicellulose A, 63100-39-0; hemicellulose B, 63100-40-3.

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Composition of the Essential Oil of Alfalfa

Takayasu Kami

The essential oil of alfalfa was isolated by steam distillation of the fresh grass with a yield of 0.009%. The essential oil was analyzed by combined gas chromatography–mass spectrometry and gas chromatographic comparison with authentic specimens, and 67 compounds consisting of hydrocarbons, aldehydes, ketones, alcohols, esters, acids, phenol, lactones, sulfur compounds, and miscellaneous were identified. Quantitative analysis was further carried out on the essential oil, and alfalfa oil was relatively rich in esters and alcohols.

In course of a series of studies on the aromatic constituents of forage crops, the present work analyzed the essential oil of alfalfa by means of combined gas chromatography–mass spectrometry and gas chromatography, following that of the previous papers (Kami, 1975, 1977, 1978, 1981) on the analyses of the essential oils of Hybridsorgo, Sudangrass, red and Ladino white clovers, and soiling dent corn. Some studies on the volatile components of alfalfa flowers (Loper et al., 1971) and of alfalfa leaves and stems (Buttery and Kamm, 1980) had been carried out so far.

Since alfalfa (*Medicago*) occupies an important role for the nutrition of domestic animals as the leguminous pasture plants, the different species of alfalfa are widely cultivated over the world. In the southwestern warm district of Japan, however, alfalfa is not cultivated so much because of inadaptation to the climate and soil of this district situated in the monsoon zone. Therefore, alfalfa is imported into Japan in the form of alfalfa pellets or alfalfa hay cubes.

EXPERIMENTAL SECTION

Materials. Alfalfa (Du Puits, *Medicago sativa* L.) was cultivated on a farm of Hiroshima Agricultural College, and the aerial parts were harvested in June 1980 by sickle. The harvest time corresponded to the flowering stage of alfalfa.

Isolation of the Essential Oil. The fresh aerial parts with flowers (230 kg), after being cut by a cutter, were steam distilled in 25-kg lots for 1 h under 0.7 kg/cm^2 distillation kettle pressure by using a boiler in a cannery of the Faculty of Applied Biological Science, Hiroshima University, and yielded about 180 L of cloudy distillate in a water-cooled trap and 28.4 g of colorless aqueous condensate in an ice–water-cooled trap. After the cloudy distillate of the water-cooled trap was saturated with sodium chloride, 2-L lots were extracted twice with 300 mL of distilled diethyl ether in the same way as described in a previous paper (Kami, 1975) to yield an yellow-brown oil with a grassy-smelling odor (21.8 g). The essential oil was stored in a sealed glass tube at 3 °C, as was the aqueous condensate from the ice–water-cooled trap.

Fractionation of the Essential Oil. A portion of the essential oil was sequentially shaken with 5% sulfuric acid, 5% sodium carbonate, and 5% sodium hydroxide aqueous solutions, 3 times with each 20 mL. The pH of each aqueous solution was reversed, and each solution was reextracted with diethyl ether to obtain basic (55 mg, 3.2%), acidic (343 mg, 20.0%), and phenolic (37 mg, 2.2%) fractions, respectively. The remaining neutral oil layer was successively extracted in *n*-pentane, diethyl ether, and diethyl ether–methanol (90:10) with silicic acid to separate

Faculty of Applied Biological Science, Hiroshima University, 2-17 Midorimachi, Fukuyama 720, Japan.

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