Separation and Partial Purification of Wax and Fatty Alcohol from Okinawan Sugar Cane Rind Lipids

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Summary A composition analysis of Okinawan sugar cane rind materials was conducted, and it was found that the main components were lipids, crude fiber and water indicating about 27%, 19.6% and 14.6%, respectively. The lipids were effectively extracted from rind materials by benzene. Wax (18%) and fatty alcohol (8%) were found to be the main components, totaling up to 96% of the lipids. Separation and partial purification of wax and fatty alcohol were attempted by means of silica gel column chromatography, thin-layer chromatography (TLC), infrared (IR) absorption spectra and gas-liquid chromatography-mass spectrometry (GC-MS). The major components of the wax were considered to be two compounds with the molecular weights of 408 (C-26) and 436 (C-28), and those of fatty alcohol were di-ol with molecular weights of 440 (C-29) and 468 (C-31). Both wax and fatty alcohol were purified by rechromatography on a silica gel column and the samples obtained seemed to be satisfactory for use in experimental rats' diets.

Key Words sugar cane rind, cane wax, cane fatty alcohol

In the previous paper (1) we reported that sugar cane rind materials showed effects on weight control and on lowering serum cholesterol levels of rats. It is widely known that the plants, generally leaves and stems, are covered with wax or some other lipids for protective purposes.

Yomo et al. (2) reported that the protecting elements of sugar cane rind such as wax and fatty alcohol were found on the surface of sugar cane, and were also mixed in the cane juice when sugar cane is pressed. In the processing of sugar cane to manufacture refined sugar, some portion of the wax and fatty alcohol remains up to the stage of raw (black) sugar.
According to the literature (3), Avequin first separated sugar cane wax in 1841 from the whitish materials attached to the surface of sugar cane. Industrial recovery of sugar cane wax had first been demonstrated in South Africa during the period of the First World War by means of solvent extraction. However, the lipid analysis of sugar cane rind materials for the purpose of finding the element(s) which serve a lowering effect on serum and (or) liver cholesterol in rats had not been attempted before this experiment.

The purpose of the present study was to analyze the main components of sugar cane rind materials and to investigate in detail the separation and partial purification of wax and fatty alcohol from sugar cane rind materials as well as to determine the molecular weight and the number of carbon atoms in the molecule.

MATERIALS AND METHODS

Materials. The sugar cane (Kind, NCO-310; Brix, 21%) used was obtained from the Experimental Farm attached to the Agriculture Department, University of the Ryukyus. The rind materials were collected (1) in two different seasons; one in January, the harvesting season, and the other in August, 6 months after planting.

Quantitative analysis of water and crude fiber. The water content was determined by a drying method (4) by using an IR moisture balance and crude fiber was measured by the A.O.A.C. gravimetric method (5).

Lipid extraction from sugar cane rind materials (6). Three different kinds of solvents; chloroform : methanol (2 : 1, v/v), benzene and n-hexane, were used for the extraction of lipids from sugar cane materials. Each solvent was added to a final dilution 20-fold the volume of the sample, and incubated at 40°C for 30 min. The solvent was immediately filtered through fat-free paper and was vaporized under a stream of nitrogen.

Column chromatography (7, 8). The glass column (2.3 × 9.5 cm) packed with silica gel (over 100 mesh, Kanto Chemical Co., Ltd.) was first washed with benzene and benzene-dissolved lipid extracts were eluted. Wax, fatty alcohol and other lipids were successively eluted with benzene, chloroform and methanol.

Thin-layer chromatography (TLC) (9). The thin-layer plate (0.25 cm thick) of silica gel G (Merck) was used, and cane wax and fatty alcohol were developed with two different kinds of solvents, petroleum ether : ethyl ether : acetic acid (92 : 8 : 1, v/v/v) at 17°C and n-hexane : benzene (1 : 1, v/v) at 30–32°C. For detection, 50% sulfuric acid was used and the plates were heated at 180°C for 15 min.

Infrared (IR) absorption spectra (10). IR absorption spectra were recorded with a HITACHI 295 spectrometer and chloroform was used as the solvent.

Gas-liquid chromatography-mass spectrometry (GC-MS) (11, 12). GC-MS was performed on a Hitachi M-80 gas chromatograph-mass spectrometer system. A glass column (200 × 0.3 cm i.d.) packed with 2% silicon OV-1 on Chromosorb WAW, DMCS (80–100 mesh) was run at 180°C (column temperature) and 280°C (injection temperature) with a helium flow of 40 ml/min. Mass spectrometry was
RESULTS

Water and crude fiber contents
The water content of sugar cane rind materials collected in January was slightly lower than that of the August sample, but the crude fiber content was just the opposite. The average contents of water and crude fiber were 14.6% and 19.6%, respectively.

Extraction of lipids
The total lipids, fatty alcohol and wax contents of sugar cane rind materials extracted by different solvents are shown in Table 1. As Paturau (6) demonstrated for the extraction of sugar cane wax, benzene was the most effective among the three solvents in each lipid showing about 27% for total lipids, 18% for wax and 8% for fatty alcohol. The solvent used by Folch et al. (13), most generally used for the extraction of lipid from animal tissues (chloroform : methanol mixture), was the next most effective and n-hexane was not adequate for effective extraction.

Separation and preparation of wax and fatty alcohol
In order to separate wax and fatty alcohol from lipids extracts, silica gel column chromatography was applied and the fractions obtained were qualitatively tested on TLC. For developing, n-hexane : benzene (1:1, v/v) was used as the solvent. As shown in Fig. 1, wax was eluted with benzene (fractions 1–4), fatty alcohol with chloroform (fractions 7–9) and other lipids with methanol (fractions 10–13). Therefore, the fractions containing wax (1–4) were combined as the total wax fraction, F-1, and concentrated for rechromatography on a silica gel column. The same procedure was done for fatty alcohol fractions (7–9), fraction F-2. As shown in Fig. 2, separated wax (F-1) and fatty alcohol (F-2) obtained by rechromatography on a silica gel column seemed to be purified enough to feed the rats for the experiment. Then, confirmation of those two fractions, F-1 and F-2, was

Table 1. Extraction of lipids a from sugar cane rind with different solvents.

| Solvents       | Total lipids (per cent/sugar cane rind b) | Fatty alcohol | Wax  |
|----------------|------------------------------------------|--------------|------|
| Chloroform:    | 25.16                                    | 5.76         | 12.18|
| methanol (2:1) |                                          |              |      |
| n-Hexane       | 16.17                                    | 4.55         | 7.91 |
| Benzen         | 26.63                                    | 8.18         | 18.28|

a Measured by weight. b Collected in January.

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Fig. 1. Thin-layer chromatogram of lipid extract of sugar cane rind separated by silica gel column chromatography. Each fraction eluted with benzene, chloroform and methanol was spotted on a thin-layer plate using silica gel G and developed with n-hexane : benzene (1:1, v/v). Wax and fatty alcohol were tentatively identified by comparing their $R_f$ values with the reported values (11).

Fig. 2. Thin-layer chromatogram of wax and fatty alcohol fractions which were purified by rechromatography on silica gel column.

Fig. 3A. Wax spot clearly identified at $R_f$ value of 0.85.

Fig. 3B. Same sample developed with n-hexane : benzene (1:1, v/v) at 30-32°C. Wax spot detected at almost the same $R_f$ value of 0.85.

The TLC was attempted by means of TLC using petroleum ether : ethyl ether : acetic acid (92:8:1, v/v/v) as developing solvents at 17°C. As shown in Fig. 3A, a wax spot was clearly identified but the fatty alcohol spot was detected at a very close $R_f$ value to that of palmitic acid. Therefore, the same samples were developed with a different solvent, n-hexane : benzene (1:1, v/v), at 30-32°C. As shown in Fig. 3B, a wax spot was detected at almost the same $R_f$ value of that previously developed and a fatty acid was also identified at a $R_f$ value of 0.85.

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Fig. 3. Identification of wax and fatty alcohol by thin-layer chromatography. A: Developed with petroleum ether:ethyl ether:acetic acid (92:8:1, v/v/v) at 17°C. B: Developed with n-hexane:benzene (1:1, v/v) at 30-32°C. 1, triolein; 2, palmitic acid; 3, fatty alcohol; 4, wax. Triolein and palmitic acid were obtained commercially. Wax and fatty alcohol were from F-1 and F-2, respectively (See Fig. 2).

Fig. 4. IR spectra of standard fatty alcohol and sugar cane rind fatty alcohol. A: C_{16} 1-hexadecanol (cetyl alcohol). B: Sugar cane rind fatty alcohol.

Fig. 5. IR spectra of sugar cane rind fatty alcohol. A: Five-fold magnification of chart B.

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alcohol spot also was clearly identified at a higher $R_f$ value than that of palmitic acid. Confirmation of the samples was, furthermore, carried out by means of IR absorption spectra to ascertain the existence of the hydroxyl radical (–OH) and ester linkage. As shown in Figs. 4–6, –OH was observed on the fatty alcohol IR spectrum at 3,200–3,500 kayser and the ester linkage on wax at 1,740 kayser.

**Determination of the molecular weight and the number of carbon atoms**

The GC chromatograms of wax and fatty alcohol are shown in Figs. 7 and 8. In regard to the wax, two major peaks and four minor peaks were observed at retention times of 117 s, 81 s, 50 s, 159 s, 203 s and 20 s in decreasing order. For the fatty alcohol, the major peak was observed at a retention time of 106 s and the
The mass spectral data of wax (two major peaks) and fatty alcohol are shown in Figs. 9 and 10. The results indicate that the wax contained two major components which displayed molecular ion peaks at \( m/e \) 408 (\( M^{-18} \)) of scan 81 and 463 (\( M^{-18} \)) of scan 117. The first component was considered to be a Carbon-26 compound with a molecular weight of 408 and the latter to be a Carbon-28 one with a molecular weight of 436. The fatty alcohol contained the two components showing molecular ion peaks at \( m/e \) 440 (\( M^+ \)) and 467 (\( M^+ \)), respectively. The first component was
Fig. 10. Gas chromatography-mass spectral data of sugar cane fatty alcohol. A. Scan: 73 RT (s): 256.7. B. Scan: 106 RT (s): 374.2.

Table 2. Fatty alcohol and wax contents of sugar cane.

| Part of sugar cane | Percentage composition |
|--------------------|------------------------|
|                    | Fatty alcohol | Wax |
| Upper (Green part) | 14            | 1   |
| Middle             | 85            | 4   |
| Lower (Mature part)| 1            | 95  |

Table 3. Comparison of fatty alcohol and wax contents of sugar cane rind.

| Samples | Lipids | Fatty alcohol | Wax |
|---------|--------|---------------|-----|
| A       | 27     | 8             | 18  |
| B       | 29     | 18            | 9   |

A: Obtained from mature sugar cane in Jan. B: Obtained from premature sugar cane in Aug.

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considered to be $C_{29}H_{58}(OH)_2$ with a molecular weight of 440, and the second, the major component, to be $C_{31}H_{62}(OH)_2$ with a molecular weight of 468.

Wax and fatty alcohol content of sugar cane rind

As shown in Table 2, the ratio of wax and fatty alcohol contained in sugar cane rind differed in direct correlation of the degree of cane-growth. Those collected from the middle to the upper part (green part) of the sugar cane contained mainly fatty alcohol whereas the lower part (mature part) were covered mostly with wax. Therefore, the sample collected in January (mature cane) contained twice as much wax as the sample collected in August (premature cane), as shown in Table 3.

DISCUSSION

For the extraction of lipids from sugar cane rind materials, benzene, which Paturau (6) previously used for extraction of sugar cane wax, was a more effective solvent than the chloroform-methanol mixture; the generally applied solvent. Therefore, the main components of the sugar cane rind lipids are now considered to be wax and fatty alcohol. The total lipid content of the January sample was about 27% of the total rind materials, and the wax and fatty alcohol contents were about 18% and 8%, respectively, composing about 96% of the total lipid extracts.

In order to separate fatty alcohol and wax from lipid extracts, column chromatography was applied as Carroll and Serdarerich (7) and Rouser et al. (8) demonstrated, and samples were eluted with $n$-hexane, benzene, chloroform and methanol successively. Judging from the $R_f$ values of each fraction obtained by TLC analysis, wax was separated by benzene, fatty alcohol by chloroform and other lipids by methanol. Those results agreed with the $R_f$ values reported by Hashimoto et al. (14) and Malins and Mangold (15).

Partial purification of fatty alcohol and wax was effectively carried out by rechromatography of each obtained fraction on a silica gel column.

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