Isolation of Methyl Ferulate From Rice Bran Oil

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ABSTRACT

An unknown ferulate in rice bran oil was isolated by column chromatography. The ferulate was identified as methyl ferulate by TLC, GLC, UV, IR, NMR and mass spectrometry.

INTRODUCTION

In 1954, Kaneko and Tsuchiya separated a new compound (mp 138.5 C) from rice bran oil, which showed three absorption maxima at 231, 291 and 315 mμ; they named it oryzanol (1).

Oryzanol was considered to be a pure compound at first. However, further investigation has shown that the compound was a mixture of two or more ferulic acid esters of triterpenoid alcohols (2). Cycloartenyl ferulate, $C_{40}H_{58}O_4$ and 24-methylenecycloartanyl ferulate, $C_{41}H_{60}O_4$ (3), steryl ferulate, $C_{38}H_{56}O_4$ (4) and β-sitosteryl ferulate, $C_{39}H_{58}O_4$ (5) were found in rice bran oil.

Recently, the determination of ferulate by reversed phase thin layer chromatography (TLC) was studied and a spot of an unknown ferulate was found (6).

This paper reports isolation and identification of a new ferulate in a dark oil from a soapstock of rice bran oil. Also the new ferulate in crude rice bran oils was determined by a combination of TLC and gas liquid chromatography (GLC).

EXPERIMENTAL PROCEDURES AND RESULTS

UV spectra were determined in *n*-heptane with a Beckman Model DU spectrophotometer. IR analyses were done on samples pelleted with KBr using a Hitachi Model EPI-S2 spectrophotometer. NMR spectra were obtained with a Japan Electron Optics Laboratory C-60HL spectrometer in deuteriochloroform solutions containing tetramethylsilane as the internal reference. Mass spectral data were determined with a Consolidated Electrodynamics Corp. mass spectrometer 110B.

Isolation of an Unknown Ferulate From Dark Oil of Rice Bran Oil

A ferulate fraction was separated from the dark oil of rice bran oil with a 5 X 67.5 cm column of 100 mesh silica gel (Kanto Chemical Corp., Tokyo). The separation was repeated in batches of 200 g of the oil with the solvent system diethyl ether-*n*-hexane (5:95 and 30:70 v/v) three times. The ferulate fraction was detected by irradiation with UV light on the silica gel column. The ferulate fractions with blue fluoresce on the column were combined by eluting with diethyl ether-hexane (30:70 v/v). An unknown ferulate was separated from the ferulate fraction with a 5 X 66 cm column of 100 mesh silica gel containing 10% water and with the elution by diethyl ether-hexane (300 ml of 10:90, 350 ml of 20:80 and 1,750 ml of 30:70 v/v).

A fraction A of the chromatogram fluorescing with light bluish purple in UV light was eluted with diethyl ether-hexane (30:70 v/v). The content of methyl ferulate in the fraction was determined by UV spectroscopic analysis. The content of methyl ferulate, 0.01% in the original dark oil was calculated by the following equation. Methyl ferulate, % = [(E X W)/(W_o X 95.6)] X 100. E, specific extinction coefficient, $E^{g/l}_{1\,cm}$ of the fraction A in *n*-heptane at 315 mμ; W, weight of fraction A, g; W_o, weight of original dark oil, g; 95.6, specific extinction coefficient, $E^{g/l}_{1\,cm}$ of methyl ferulate at 315 mμ.

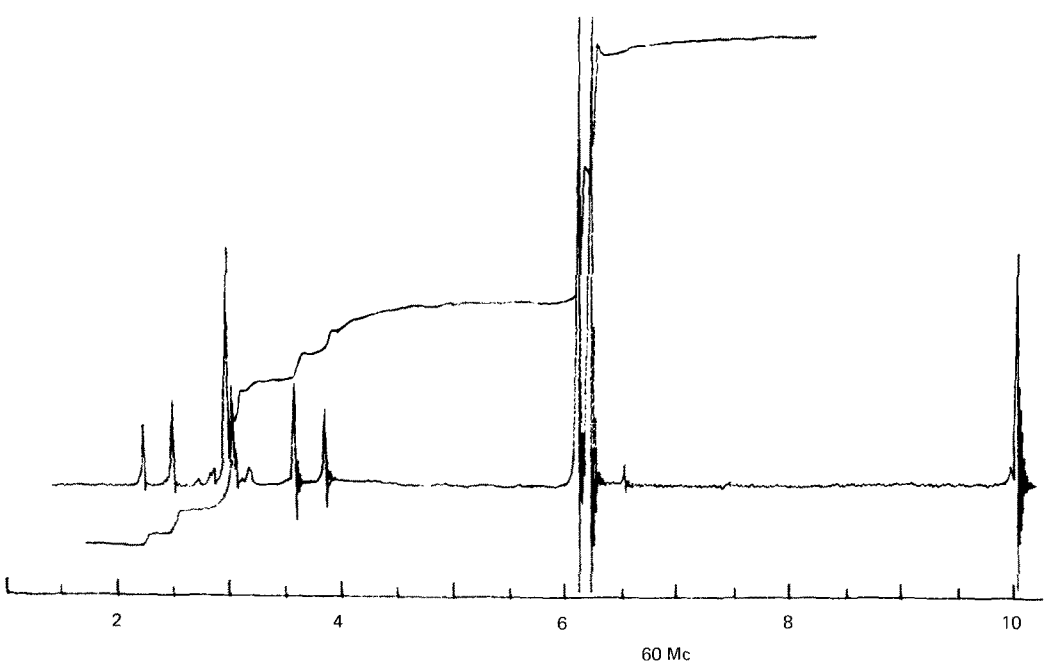

FIG. 1. NMR spectrum of crystal A_2.

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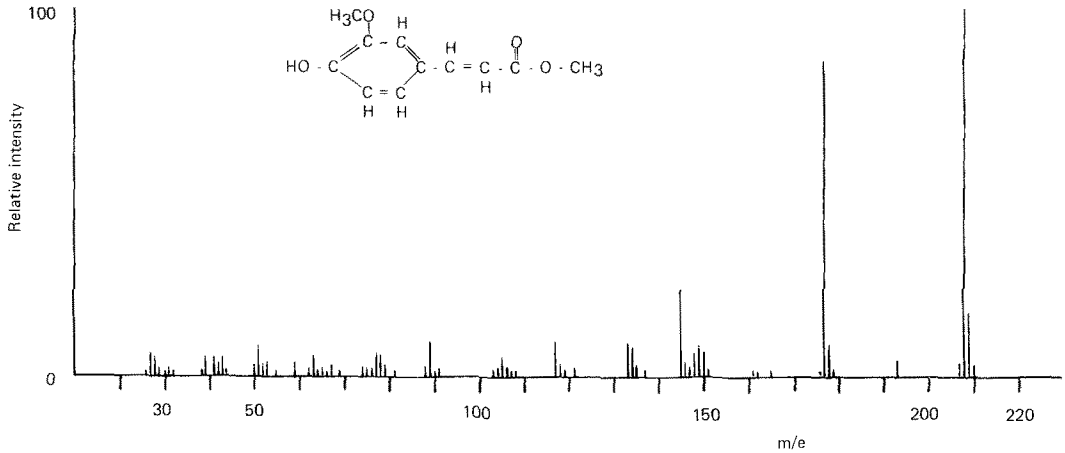

FIG. 2. Mass spectrum of crystal A_2 at 70 eV.

Isolation of Fraction A_1

Fraction A_1 was separated from other constituents by chromatographing the fraction A on a 2.5 X 38.5 cm column of 100 mesh silica gel. At the start of the run the solvent was 10% diethyl ether in hexane. The diethyl ether content was increased in increments of 10% until the fraction A_1 was finally eluted with 30% diethyl ether in hexane.

Refluxing 110 mg of the fraction A_1 with 15 ml of acetic anhydride-pyridine (2:3 v/v) for 1 hr, yielded 115 mg of an acetate of fraction A_1. The acetate was crystallized in chloroform-ethanol (1:3 v/v). The acetate had mp 120 C and λ_{max} 225 mμ ($E^{g/l}_{1cm}$ 60.2), 277 mμ ($E^{g/l}_{1cm}$ 77.1) and 308 mμ ($E^{g/l}_{1cm}$ 42.6).

The acetate was treated with N/10 potassium hydroxide in ethanol at 50 C to convert into the original ferulate. The converted ferulate was crystallized in ethanol-hexane, (1:15 v/v). The crystal A_2, 55 mg was mp 64 C and λ_{max} 231 mμ ($E^{g/l}_{1cm}$ 65.1), 291 mμ ($E^{g/l}_{1cm}$ 78.9) and 315 mμ ($E^{g/l}_{1cm}$ 95.6). The NMR spectrum of crystal A_2 revealed equivalent numbers of two methoxyl (6.21 and 6.11 τ), and aromatic (2.97 τ) protons (Fig. 1).

Synthesis of Methyl Ferulate

Methyl ferulate was synthesized from ferulic acid and methanol by Pearl and Beyer's method (7) and purified by column chromatography and crystallization from ethanol-hexane (1:15 v/v), mp 64.3 C (lit. value 65.0 C, Ref. 8).

Analysis calculated for $C_{11}H_{12}O_4$: C, 63.46%; H, 5.81%. Found: C, 63.40%; H, 5.67%.

The ferulate was converted to acetylferulate by refluxing for 2 hr with acetic anhydride-pyridine, mp 120.7 C (lit. value 123.0 C, Ref. 8).

Analysis calculated for $C_{13}H_{14}O_5$: C, 62.39%; H, 5.64%. Found: C, 62.44%; H, 5.42%.

Identification of Crystal A_2 by TLC, GLC, UV, IR and NMR

Analyses of the synthetic methyl ferulate and of crystal A_2 by TLC were done on plates with 250 μ layers of Kieselguhr B-10 (Wako Pure Chem. Ind. Ltd., Tokyo) which was impregnated with paraffin oil by dipping it into a 10% solution of paraffin oil in petroleum ether. After the impregnated plate was dried, hexagonal holes were brushed out of the kieselgur layer on the plate to provide five chromatograms modelled on Matthias's technique (9). Chloroform solutions of the synthetic methyl ferulate and of crystal A_2 were spotted in a line on the plate prepared by the above method. The plate was developed with methanol-acetonitrile (2:1 v/v). The spots of synthetic methyl ferulate and crystal A_2 showed the same R_F 0.93 on the plate by irradiation with UV light.

Trimethylsilyl ethers of the synthetic methyl ferulate and crystal A_2 respectively were prepared by warming approximately 5 mg of sample and 1 ml of a commercially prepared mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine (Trimethylsilyl Ether Kit, Shimadzu Seisakusho Ltd., Kyoto, Japan) at 50 C for 10 min. Gas chromatography of the trimethylsilyl ether in diethyl ether was conducted on a Shimadzu Model 1 C gas chromatograph with a hydrogen flame detector using a 1.5 m long, 3 mm O.D. stainless steel column packed with 3% SE-30 on 60-80 mesh Chromosorb W AW. Injection port temperature was 220 C, and the column was operated at 180 C with helium carrier gas flow-rate of 60 ml/min. The trimethylsilyl ethers of the synthetic methyl ferulate and crystal A_2 showed the same retention time, 16.0 min. In addition, the UV, IR and NMR spectra of the synthetic methyl ferulate were identical to these of crystal A_2.

Mass Spectra of Crystal A_2 and Methyl Ferulate

The pattern shown in Figure 2 for crystal A_2 is characterized by a peak (loss of two methoxy radicals and hydrogen from methyl ferulate) at m/e 145, a peak (loss of methoxy radical from methyl ferulate) at m/e 177 and a parent peak at m/e 208. These portions of the fragmentation patterns for crystal A_2 were identical to these of methyl ferulate.

Detection of the New Ferrulate in Fraction B_1, B_2 and B_3 From the Three Kinds of Rice Brain Oils

Rice brans from three kinds of rice grown in Japan (Chiba, Saitama and Shizuoka) were extracted with diethyl ether and the methyl ferulate fraction B_1, B_2 and B_3 in the three extracted oils were separated by silica gel-column chromatography by the same methods as for the separation

TABLE I

Rice Bran Oils

Geographical source	Chiba	Saitama	Shizuoka
Oil in rice bran[a], %	21.46	20.74	21.75
$E^{g/l}_{1cm}$ 315 mμ[b] on extracted oil	0.64	0.82	0.87
A new ferulate fraction	B_1	B_2	B_3
Extracted oil[c]	0.006	0.024	0.027

[a]Air-dry. Moisture 9% to 10%.

[b]In *n*-heptane.

[c]Weight per cent of methyl ferulate in extracted oils by UV spectroscopic analysis.

of methyl ferulate in the dark oil of rice bran oil (Table I).

Analyses of the new ferulate fraction B_1, B_2 and B_3 were carried out using the same method as for the analysis of synthetic methyl ferulate and crystal A_2 by TLC and GLC. Fraction B_1, B_2 and B_3 gave respectively one spot by irradiation with UV light on TLC. The R_F values of these spots were attributed to methyl ferulate. Trimethylsilyl ethers of the fraction B_1, B_2 and B_3 were applied with the gas chromatograph and gave respectively one peak which was attributed to methyl ferulate.

DISCUSSION

The experimental work shows conclusively that methyl ferulate occurs in the dark oil from rice bran oil. Methyl ferulate is also detected in the extracted rice bran oils from three kinds of rice brans. Methyl ferulate represents 0.006-0.027% of rice bran oil.

Previously, ferulates of triterpenoid alcohols and sterols were isolated from rice bran oil (2-5). Methyl ferulate is characteristic in the structure of alcohol among these ferulates in rice bran oil. Since methyl ferulate is prepared easily by esterification of ferulic acid with methanol or interesterification of alcohol of ferulates, it is considered to be probable that methyl ferulate is formed during the process of separation from dark oil or rice bran oils if methanol is used. However, methanol was not used during the process of isolation of methyl ferulate, and so methyl ferulate was present originally in dark oil or rice bran oils.

Several other oils and oats contain other ferulates, for example dihydro-β-sitosteryl ferulate in corn oil (10), dihydro-γ-sitosteryl ferulate in wheat oil (11), and *n*-eicosanyl ferulate in oils of linseed and rapeseed (12) were reported. Further, glyceryl ferulate was contained in the antioxidants extracted from oats (13).

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