TABLE V

Ultrafiltration and Reverse Osmosis of Corn Hominy Feed Stillage Solubles[a]

	Volume, ml	Nitrogen, mg/ml	Solids, mg/ml	Ash, % of dry matter
Stillage solubles	3,040	0.759	32.08	18.67
Permeate (UF)	2,580	0.355	16.50	28.62
Concentrate (UF)	106	1.04	37.44	16.56
Permeate (RO), total	1,699	0.023	1.11	20.2
Permeate (RO), end	85	0.063	2.23	23.9
Concentrate (RO), total	634	0.513	21.91	27.9
Concentrate (RO), end	234	0.691	27.92	28.1

[a]Permeate from ultrafiltration (UF) accounts for 85% of original volume, 44% of total solids and 40% of total nitrogen. Permeate (UF) was used as feed solution for reverse osmosis (RO) after about 100 ml was removed for analyses. Permeate (RO) accounts for 70% of original volume, 5% total solids and 5% total nitrogen of permeate (UF). In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also accounted for the initial volume. The original volume of UF permeate for RO was 2,430 ml.

permeate and higher nitrogen and solids contents for RO concentrate were experienced. Reduction in nitrogen and solids contents of RO permeate was the largest, on a percentage basis, among all dry-milled corn fractions stillage solubles studied.

CONCLUSION

The combination of UF and RO processing of stillage solubles from corn grits, flour, degerminated meal and hominy feed appears to be an attractive method to separate stillage solubles into a large volume of permeate having low nitrogen and solids content and a small volume of concentrate with high nitrogen and solids contents. The RO permeate may be reused as process water, further treated, or discharged.

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REFERENCES

1. Gregor, H.P., and C.D. Gregor, Sci. Am. 239(1):112 (1978).
2. Gregor, H.P., and T.W. Jeffries, in Biochemical Engineering, edited by W.R. Vieth and K. Venkatasubramanian, Annual N.Y. Academic Science, New York, 1979, pp. 273.
3. Sheehan, G.J., and P.F. Greenfield, Water Res. 14:257 (1980).
4. Wu, Y.V., K.R. Sexson and J.S. Wall, Cereal Chem. 60:248 (1983).
5. American Association of Cereal Chemists, Approved Methods of the AACC, St. Paul, MN, 1976.
6. Wu, Y.V., K.R. Sexson and J.S. Wall, Cereal Chem. 58:343 (1981).

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♣The Structure of Rosmariquinone - A New Antioxidant Isolated from *Rosmarinus officinalis L.*

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ABSTRACT

A new diterpene, named rosmariquinone, was isolated from the leaves of *Rosmarinus officinalis L.* The leaves were first extracted using methanol and, upon further purification, this extract yielded rosmariquinone. Structure elucidation of the antioxidant was done using IR, MS, ^{1}H-NMR and ^{13}C-NMR.

INTRODUCTION

Work by Chipault et al. (1,2) described the antioxidant properties of more than 30 spices. They reported that rosemary *(Rosmarinus officinalis L.)* and sage *(Salvia officinalis L.)* had the greatest antioxidant properties of all the spices tested. The antioxidant findings concerning rosemary triggered several investigations aimed at isolating and identifying the active compounds in this spice.

The first important antioxidant compound isolated from *Rosmarinus Officinalis L.* was a phenolic diterpene named carnosol. The structure of carnosol was determined correctly by Brieskorn et al. (3). Wu et al. (4), using a different isolation method, also identified carnosol from rosemary leaves. Inatani et al. (5) isolated and characterized a new antioxidant, rosmanol, from rosemary leaves. Rosmanol is also a phenolic diterpene with a structure similar to that of carnosol. Recently, we reported (6) a novel antioxidant compound, rosmaridiphenol, from rosemary leaves. In addition to these specific compounds, several investigators (7-10) have reported preparing various extracts of rosemary which contained antioxidant activity.

This paper reports the isolation and characterization of a new antioxidant, rosmariquinone, from rosemary leaves.

EXPERIMENTAL

Extraction and Fractionation of Rosemary Leaves

A rosemary antioxidant product was prepared from ground rosemary leaves following a procedure described by Wu et al. (4). This antioxidant was fractionated by using a

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5 cm × 122 cm glass column packed with activated silicic acid (11). The column was eluted by stepwise gradient using first 100% hexane and then 5% diethyl ether in hexane (E/H), 10% E/H, 15% E/H, 25% E/H, 50% E/H, 75% E/H and, finally, 100% methanol. A total of 15 fractions resulted from this elution pattern.

Identification of the New Antioxidant

An infrared (IR) spectrum was obtained using a KBr pellet on a Beckman Acculab 4 spectrophotometer. A mass spectrum was taken using a Du Pont 21-490 mass spectrometer. The source temperature was held at 100 C with the ionization voltage at 70 ev. Both the ^{1}H and ^{13}C-NMR spectra were obtained using a Bruker 250 MH_z NMR spectrophotometer. Quantitative elemental analysis of carbon and hydrogen was performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

Antioxidant Activity Analysis

Antioxidant activity of compounds was determined by the reduction of peroxide formation in lard samples. The samples were kept at 60 C in the dark over a 4-week period. Peroxide values were determined by Official Method Cd 8-53 of the American Oil Chemists' Society (12).

RESULTS AND DISCUSSION

Isolation of the New Antioxidant

Upon chromatographic fractionation of the rosemary antioxidant, the hexane-diethyl ether 85:15 fraction contained two main components. The first was a brown, viscous liquid. Dispersed in this liquid were red crystals. The crystals were separated from the liquid portion and were recrystallized using hexane. Pure, needle-like red crystals were obtained upon recrystallization.

Identification of the New Antioxidant

Through spectroscopic methods, the red crystals isolated were identified as rosmariquinone (structure shown in Figure 1), $C_{19}H_{22}O_2$, m.p. 94-95 C. Elemental composition was calculated as 80.82% carbon, 7.85% hydrogen, and this was in good agreement with the experimental data of 81.17% carbon, 7.76% hydrogen.

The infrared spectrum of rosmariquinone exhibited aromatic C-H stretching at 3090 cm^{-1} and indicated a conjugated ketone system by the presence of a band at 1649 cm^{-1}. Other IR absorptions were at 2960, 2940, 1570, 1560, 1450, 1410, 1380, 1300, 1250, 1140 and 920 cm^{-1}.

Rosmariquinone's mass spectrum gave fragments at m/z (relative intensity) 282 (M^+, 7%), 267 (5%), 254 (52%), 239 (100%), 224 (17%), 219 (6%), 209 (9%), 197 (8%) and 165 (10%). The ion at m/z 254 strongly suggests a loss of CO while the peak at m/z 238 represents the loss of the isopropyl group from the molecular ion.

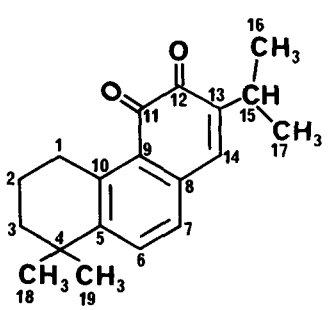

FIG. 1. Structure of Rosmariquinone.

TABLE I

^{1}H-NMR Data of Rosmariquinone

Proton No.	δ
H-1	3.17 (2H, t, J = 6.3 H_z)
H-2	1.79 (2H, m)
H-3	1.64 (2H, m)
H-6	7.60 (1H, d, J = 8.0 H_z)
H-7	7.12 (1H, d, J = 8.0 H_z)
H-14	7.09 (1H, s)
H-15	3.02 (1H, sept, J = 7.1 H_z)
H-16 and 17 (Me)	1.17 (6H, d, J = 6.8 H_z)
H-18 and 19 (Me)	1.30 (6H, s)

The proton NMR spectrum of rosmariquinone, listed in Table I, shows a doublet at δ1.17 corresponding to the protons of the methyl group of the isopropyl side chain, and a singlet at δ1.30 indicating the protons of the two methyl group substituents of the aliphatic ring. Also, a septet appears at δ3.02 corresponding to the methine proton of the isopropyl groups. In addition, the three aromatic protons give peaks at δ-7.09, 7.12 and 7.60. The other aliphatic protons appear as multiplets between δ1.5-3.2.

The carbon-13 NMR spectrum of rosmariquinone (Table II) shows 17 carbon atom signals; however, it should be recalled that rosmariquinone contains 19 carbon atoms. The peaks at 31.8 and 21.5 ppm are actually two sets of two equivalent atoms. The peak at 21.5 ppm corresponds to the two methyl carbons of the isopropyl unit. The signal at 31.8 ppm shows the two carbons of the methyl substituents of the aliphatic ring. The peak at 26.9 ppm represents the methine carbon of the isopropyl group. The two carbonyl carbons of the o-quinone ring appear at 181.5 and 182.4 ppm. The eight aromatic carbon atoms appear as signals between 127.9-149.7 ppm. The off-resonance ^{13}C-NMR spectrum indicated that the following groups are present in rosmariquinone: four methyl groups, three methylene groups, one methine group, one quaternary carbon atom, two carbonyl groups and eight aromatic carbon atoms of which three possess a carbon-hydrogen bond.

All of the spectroscopic data obtained are in agreement with the proposed structure of rosmariquinone.

Antioxidant Activity of Rosmariquinone

Rosmariquinone was tested for antioxidant activity in

TABLE II

^{13}C-NMR Chemical Shifts of Rosmariquinone

Carbon No.	Chemical shift (ppm)
C-1	29.9 (t)
C-2	19.1 (t)
C-3	37.8 (t)
C-4	34.5 (s)
C-5	144.5 (s)
C-6	133.8 (d)
C-7	139.9 (d)
C-8	128.2 (s)
C-9	134.4 (s)
C-10	145.0 (s)
C-11	182.4 (s)
C-12	181.5 (s)
C-13	149.7 (s)
C-14	127.9 (d)
C-15	26.9 (d)
C-16 and C-17	21.5 (q)
C-18 and C-19	31.8 (q)

TABLE III

Antioxidant Activity of Rosmariquinone and Other Commercial Antioxidants

Additive (0.02%)	Peroxide value (Meq/kg) at 60 C after days: 7	14	21	28
Control, no additive	4.70	10.08	29.93	119.67
BHT	1.26	1.86	2.71	3.37
BHA	2.72	6.54	12.10	17.01
Rosmariquinone	3.28	3.81	4.52	5.10

prime steam lard at a concentration of 0.02%. The peroxide value of the lard was determined when the samples were fresh and after being aged at 60 C in the dark for 7, 14, 21 and 28 days, respectively. The decrease in the rate of formation of peroxide was used as a measurement of the antioxidant activity of the sample. The peroxide values obtained from rosmariquinone and other standards appear in Table III. The antioxidant activity of rosmariquinone was superior to BHA, but it was slightly less than BHT.

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REFERENCES

1. Chipault, J.R., G.R. Mizuno, J.M. Hawkin and W.O. Lundberg, Food Res. 17:46 (1952).
2. Chipault, J.R., G.R. Mizuno and W.O. Lundberg, Food Tech. 10:209 (1956).
3. Brieskorn, C.H., A. Fuchs, J.B. Bredenberg, J.D. McChesney and E. Wenkert, J. Org. Chem. 29:2293 (1964).
4. Wu, J.W., M.-H. Lee, C.-T. Ho and S.S. Chang, JAOCS 59:339 (1982).
5. Inatani, R., N. Nakatani, H. Fuwa and H. Seto, Agric. Biol. Chem. 46:1661 (1982).
6. Houlihan, C.M., C.-T. Ho and S.S. Chang, JAOCS 61:1036 (1984).
7. Rac, M., and B. Ostric, Rev. Franc. Corp. Gras. 2:796 (1955).
8. Berner, D.L., and G.A. Jacobson, U.S. Patent 3,732,111 (1973).
9. Chang, S.S., B. Ostric-Matijasevic, O.A.-L. Hsieh and C.-L. Huang, J. Food Sci. 42:1102 (1977).
10. Bracco, U., J. Löliger and J.L. Viret, JAOCS 58:686 (1981).
11. Sahasrabudhe, M.R., and D.G. Chapman, JAOCS 38:88 (1961).
12. Official and Tentative Methods of the American Oil Chemists' Society, Third Edition, American Oil Chemists' Society, Champaign, IL (1973).

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Comparative Studies of Three Solvent Mixtures for the Extraction of Soybean Lipids

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ABSTRACT

Soybean seeds were extracted with chloroform-methanol (2:1), methylene chloride-methanol (2:1) and hexane-isopropanol (3:2) mixtures. The seed lipids were then fractionated by column chromatography. Neutral lipids were further separated by thin layer chromatography (TLC) and quantified by acid charring method. Fatty acid methyl esters were prepared and analyzed by gas liquid chromatography (GLC). Our results show that methylene chloride-methanol (2:1) was as good as chloroform-methanol (2:1) for the extraction of soybean lipids, but hexane-isopropanol (3:2) was somewhat inferior.

INTRODUCTION

Chloroform-methanol (2:1) mixture has been widely accepted as the most exhaustive solvent mixture and has been used extensively for the extraction of plant and animal lipids (1). However, the extensive usage of chloroform has caused great concern recently because chloroform is hepatoxic and a suspected carcinogen (2,3). In view of this, two other solvent mixtures, hexane-isopropanol (3:2) (4) and methylene chloride-methanol (2:1) (5), which avoid the use of chloroform, recently have been suggested. These new solvent mixtures have not been widely tested under laboratory conditions. In this communication, we compare the effectiveness of the three solvent mixtures for the extraction of soybean seed lipids under the same laboratory conditions.

EXPERIMENTAL

Materials

Soybean seeds were obtained from Lam Soon Oils and Soaps Manufacturing Limited, Petaling Jaya, Selangor, Malaysia. The seeds were ground mechanically to a fine powder before lipid extraction.

All organic solvents were of analytical grade, and chloroform was redistilled before use.

Methods

Lipids were extracted with chloroform-methanol (2:1) according to Folch et al. (6), with methylene chloride-methanol (2:1) according to Chen et al. (5) and hexane-isopropanol (3:2) according to Hara and Radin (4). Total lipids were fractionated into neutral lipid, glycolipid and phospholipid fractions by HCl-treated Florisil column chromatography (7). Neutral lipids were separated further by TLC using hexane-diethyl ether-formic acid (80:20:2) as the developing solvent system and quantified by sulphuric acid charring method (8). Fatty acid methyl esters (FAME) were prepared and analyzed as described before (9).

RESULTS AND DISCUSSION

Our results (Table I) show that chloroform-methanol (CM) and methylene chloride-methanol (MM) extracted comparable amounts of total seed lipids, whereas hexane-isopropanol (HI) extracted much less seed lipids. The difference between the MM and HI extracted seed lipids is statistically significant ($P<0.05$).

Fractionation of the soybean total lipids in HCl-treated Florisil column showed that the HI method extracted much less phospholipids compared to those extracted by the CM and MM methods (Table I). Sahasrabudhe and Smallbone (10) also reported that the HI method extracted much less polar lipids from beef compared to the CM and MM methods.

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