Nonsaponifiable Lipid Constituents of Cardamom

M. Gopalakrishnan,*,‡ C. S. Narayanan,‡ and Michael Grenz§

Regional Research Laboratory (CSIR), Trivandrum 695019, India, and Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, FRG

A chemical investigation based on ^1H NMR and MS studies revealed that the nonsaponifiable lipid fraction of cardamom consisted mainly of waxes and sterols. The waxes identified were *n*-alkanes (C_{21}, C_{23}, C_{25}, C_{27}, C_{29}, C_{31}, and C_{33}) and *n*-alkenes (C_{21}, C_{23}, C_{25}, C_{27}, C_{29}, C_{31}, and C_{33}). In the sterol fraction β-sitostenone and γ-sitosterol are newly reported. Phytol and traces of eugenyl acetate were also identified in cardamom for the first time.

INTRODUCTION

Cardamom (*Elettaria cardamomum* Maton, var. miniscula Burkill) is an important spice noted for its delicate aroma and flavor. In medicine it is used as a stimulant, carminative, stomachic, and diuretic. The volatile oil of the spice (8–10%), which imparts the flavor and aroma of the spice, has been studied in detail in the past (Purseglove et al., 1981). The spice also contains a fixed oil (1–10%), which is supposed to have a twofold function viz. to act as a fixative for the aroma and to contribute to the total flavor/physiological properties. The fatty acid part of the fixed oil has been studied and reported by a few workers (Kasturi and Iyer, 1955; Miyazawa and Kameoka, 1975; Salzer, 1975; Marsh et al., 1977), but the nonsaponifiable fraction has not been studied until recently. The nature of the glycolipids (8.7%) and phospholipids (1.9%) in the cardamom seeds has been described in a recent paper (Kotaoka et al., 1987). The phospholipid fraction consisted of cardiolipin, phosphatidylethanolamine, phosphatidylinositol, and lysophosphatidylcholine. The sterol components were reported to be 7-ergosterol and campesterol. In addition, γ-tocopherol was also reported to be present in the lipid fraction of cardamom. More recently, a few more sterols (desmosterol, β-sitosterol, stigmasterol, $\Delta^{5,7,22}$-cholestatrienol and cholesterol), α-tocopherol, and a few saturated hydrocarbons (C_{22}, C_{24}, C_{25}, C_{26}, C_{28}, C_{29}, C_{30}, C_{33}) were also reported to have been identified on the basis of TLC and GC analysis (Shaban et al., 1988). The present paper is based on a chemical investigation by ^1H NMR and MS carried out on the nonsaponifiable fraction of the fixed oil of cardamom.

EXPERIMENTAL PROCEDURES

Materials. Fresh Alleppey green cardamom was procured from Idukki District of Kerala State (India) for the study.

The chemicals used were of analytical reagent grade quality. Laboratory-distilled solvents were used throughout the experiment. Authentic samples were either procured from reputed firms or prepared in the laboratory. For column chromatography silica gel 60–120 mesh (Merck Darmstadt) was used. For thin-layer chromatography precoated silica gel sheets (aluminum) (20 cm × 20 cm, 0.25 mm layer thickness, Merck Darmstadt) were used.

Preparation of Nonsaponifiable Fraction. The volatile oil of the ground cardamom seeds (7.5 kg) was removed by steam distillation, and the residue was dried at room temperature. Hot extraction of the residue with petroleum ether (40–60 °C) yielded ~129 g of crude extract. This crude extract (128 g) was saponified as per standard procedure (Gunstone et al., 1986) to yield ~66 g of fatty acids and ~11.9 g of nonsaponifiable fraction (NSF).

Separation of Waxy Materials. The NSF (7.3 g) was made to dissolve in methanol (500 mL), and the undissolved part (~5.5 g) was filtered off (fraction A). The soluble fraction B was kept in a refrigerator overnight. The solid waxy material (fraction B_1, ~1.5 g) was separated by quick filtration. The cold odorous methanol fraction was concentrated to get an oil (fraction B_2, ~0.3 g). The solid fraction B_1 was passed through a bed of silica gel (60–120 mesh) by elution with hexane to obtain a waxy hydrocarbon fraction B_{1a} (~0.5 g). The polar compounds on the silica gel were washed out with diethyl ether and concentrated (fraction B_{1b}, ~1.0 g).

Column and Thin-Layer Chromatography. Fraction A (3.2 g) was chromatographed on a silica gel column (60 g) using gradient elution with petroleum ether (40–60 °C)/diethyl ether in different ratios with increasing polarity, and the eluates were collected in 20-mL fractions. Fractions 1–4 were pooled together and concentrated to get a waxy solid (~1 g). Preparative TLC of the solid (silica gel/$AgNO_3$, hexane) afforded two bands (R_f 0.98 and R_f 0.96) which were extracted out in diethyl ether and analyzed by GC–MS. Direct GC–MS analysis of fractions 5–9 indicated two compounds, **2a** and **2b**, and fractions 10–12 yielded mainly a single compound, **3a**. Preparative TLC of fractions 14–20 gave three compounds, **4a–c**, and fractions 21–23 which constituted the major chromatographic fractions afforded a yellowish brown solid (~1.5 g). Preparative TLC of fractions 24–28 (silica gel, hexane/EtOAc, 80:20) gave an additional compound, **6a**.

Fraction B_{1a} resembled the waxy hydrocarbon fractions 1–4 previously described and was worked out similarly (Scheme I). The polar fraction B_{1b} resembled the polar subfractions 14–20/21–23 (from fraction A) described previously and was worked out in the same way. The liquid fraction B_2 (~0.3 g) was analyzed by GC–MS.

For preparatory TLC the bands were cut out and the compounds were extracted in diethyl ether. Visualization of the spots was generally done in UV light. Alternatively, the plates were sprayed with 50% H_2SO_4 and heated at 110 °C for half an hour and the spots observed. Silver nitrate plates were made by uniformly spraying the precoated TLC plates with saturated aqueous $AgNO_3$ solution followed by drying at 105 °C for 30 min.

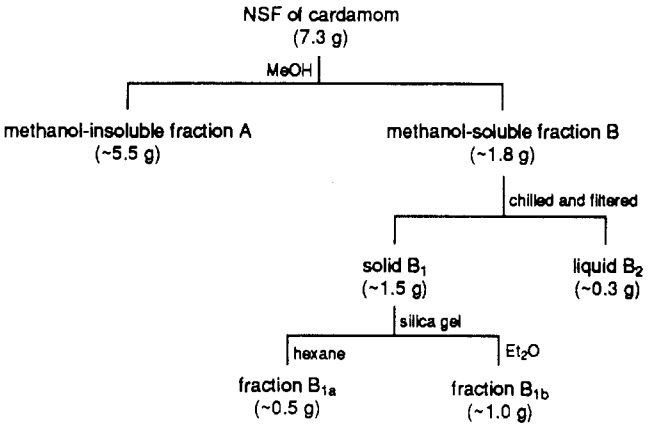
GC–MS Analysis. A Hewlett-Packard 5995 GC–MS equipped with a methylsilicone flexible silica capillary column (50 m × 0.25 mm i.d.) was used for analysis of the fractions. GC conditions were helium carrier gas (1 mL/min), split ratio 1:75, injection temperature 250 °C, and temperature programmed from 80 to 220 °C at 5 °C/min. MS conditions were electron impact, ionizing voltage 70 eV, source temperature 150 °C, analyzer at 180 °C, electron multiplier at 2000 eV, scan speed 690 amu/s, and scan range 40–500 amu.

MS of pure compounds isolated were recorded by direct probe method with temperature programmed from 100 to 300

‡ Regional Research Laboratory.
§ Technical University of Berlin.

0021-8561/90/1438-2133$02.50/0

Scheme I



°C at 10 °C/min. ^{1}H NMR spectra were recorded on a Bruker WM 400 NMR spectrometer. Samples were dissolved in $CDCl_3$ containing TMS as internal standard. IR spectra were recorded by using a Perkin-Elmer 299 B model as KBr pellets.

Preparation of Silyl Derivatives. A slight excess of BSA [*N,O*-bis(trimethylsilyl)acetamide] (Fluka AG Chemical Co., flammable, irritant to skin) in CS_2 was treated with 0.2 mg of the waxy hydrocarbon fraction (lower band, R_f 0.96) in a capped vial and heated to near boiling point (Pierce, 1968). MS of the reaction product were checked by injecting a calculated 2 μg of the product in the reaction mixture.

Oxidation of Compound 5b. To a pure sample of **5b** (0.2 mg) in dry methylene chloride was added a slight excess of pyridinium chlorochromate (Aldrich Chemical Co., cancer suspect agent, oxidizer), and the mixture was stirred at room temperature for 2 h (Fieser and Fieser, 1967). The product was purified and analyzed.

RESULTS AND DISCUSSION

The identification of compounds made during the investigation was based on mainly ^{1}H NMR (400 MHz) and MS spectra in comparison with published data (Rubinstein et al., 1976; Pouchert and Campbell, 1974; Szymanski and Yelin, 1968; Jennings and Shibamoto, 1980; Graselli and Ritchey, 1975; MSDC, 1983).

The NSF of cardamom contained a sizeable amount of resinous matter (~75%) insoluble in methanol. On column chromatography on silica gel, fraction A yielded a mixture of waxy hydrocarbons in the first four fractions (~1 g), which was analogous to the waxy hydrocarbon fraction, B_{1a}. Ion chromatogram of the total hydrocarbon fraction showed relatively minor peaks dominated by regular series of doublets. On rechromatography (silica gel/$AgNO_3$, hexane) both the fractions were separated into two bands. On GC–MS analysis, an extract of the upper band (R_f 0.98) revealed molecular ions corresponding to saturated straight-chain hydrocarbons, $C_{23}H_{48}$, $C_{25}H_{52}$, $C_{27}H_{56}$, $C_{29}H_{60}$, $C_{31}H_{64}$, and $C_{33}H_{68}$ (**1a–f**, Table I). IR and ^{1}H NMR spectra of the extract confirmed the nature of these compounds. Unlike an earlier paper (Shaban et al., 1988), predominantly odd numbered hydrocarbons were detected in cardamom in the present studies. On the other hand, ^{1}H NMR of the lower band (R_f 0.96) indicated olefinic protions. MS of the fraction indicated molecular ions corresponding to unsaturated straight-chain hydrocarbons, $C_{23}H_{46}$, $C_{25}H_{50}$, $C_{27}H_{54}$, $C_{29}H_{58}$, $C_{31}H_{62}$, and $C_{33}H_{66}$ (**1g–l**). They also showed no reaction with BSA, thereby ruling out the possibility of hydroxyl groups in the compounds. All these compounds showed characteristic MS peaks corresponding to C_nH_{2n-1} or C_nH_{2n+1} pattern separated by 14 mass units as in the case of straight-chain alkenes or alkanes, respectively, which suggested that they are probably 1-alkenes. The mass spectra of the compounds were also characterized by a smooth decrease in intensity with carbon number and maximal chain length. The presence of 1-alkenes is rarely reported in plants, and their biogenetic role is not well understood. It is postulated that they may have a role in the formation of the seed wall in cardamom.

Fractions 5–9 yielded an odourous oil in traces. GC–MS analysis of the oil (Table II) showed α-copaene (**2a**) and α-ylangene (**2b**) as the two constituents of this fraction, both of which have been reported to be present in cardamom (Lawrence et al., 1979). Fractions 10–12 also yielded an oily fraction (~30 mg) with a pleasant smell. ^{1}H NMR and MS of this fraction indicated mainly the presence of nerolidol (**3a**), which is also reported to be a constituent of cardamom oil (Purseglove et al., 1981).

Fractions 14–20 on preparative TLC (silica gel, hexane/EtOAc, 80:20) gave three spots, **4a**, **4b**, and **4c**. Analysis of **4a** did not give any conclusive evidence on its identity.

β-Sitostenone. MS of compound **4b** (R_f 0.56) showed molecular ion at m/z 412 with a characteristic peak at m/z 124 (Table III). ^{1}H NMR signal at δ 5.72 (1 H, s) was characteristic for an olefinic proton conjugated to a carbonyl group. The signals at δ 0.70 (3 H, s, C-18), 0.81 (3 H, d, C-27, overlapped), 0.82 (3 H, d, C-26, overlapped), 0.83 (3 H, t, C-29), 0.92 (3 H, d, C-21), 1.18 (3 H, s, C-19), corresponding to six methyl groups, suggested its identity as a ketosterone. The MS peak at m/z 124 was also characteristic of a keto group in the ring. The compound gave ^{1}H NMR and MS spectra identical with those of authentic β-sitostenone.

Unidentified Compound. Compound **4c** (R_f 0.53) gave molecular ion at m/z 426 and a (M − 18) peak at m/z 408. ^{1}H NMR spectrum showed a signal at δ 5.4 (1 H, br t), indicating olefinic proton. A signal at δ 4.15 (doublet) showed a hydroxymethyl group. In general, the compound appeared to be a triterpene. The identification is, however, incomplete.

Stigmasterol. Fractions 21–23 afforded ~1.5 g of a brown solid which on preparative TLC (silica gel, hexane/EtOAc, 80:20) afforded only a single band (R_f 0.51) initially but on three repeated developments gave three narrow bands, **5a**, **5b**, and **5c**. ^{1}H NMR of the upper band, **5a**, showed signals at δ 0.69, 0.79, 0.80, 0.84, and 1.01 (C-21, C-19), corresponding to six methyl groups. A multiplet at δ 3.51 (1 H) showed a β-hydroxyl group. A characteristic signal at δ 5.44 (1 H, d) indicated olefinic proton (at C-4). Another weak signal at δ 5.10 showed an additional double bond. Mass spectrum of the compound showed M^+ at m/z 412 and a (M − 18) peak at m/z 394. From the R_f value, which concurred with published information (Coscia et al., 1984), ^{1}H NMR, and MS pattern the compound was identified as stigmasterol in conformity with an earlier finding (Shaban et al., 1988).

β-Sitosterol. ^{1}H NMR spectrum of the second band, **5b**, showed signals at δ 0.68, 0.81, 0.83, 0.84, 0.92, and 1.00, corresponding to six methyl groups. A multiplet at δ 3.51 (1 H) showed a β-hydroxyl group. A characteristic signal at δ 5.34 (1 H, d) indicated an olefinic proton. Mass spectrum of the compound showed M^+ at m/z 414 and a (M − 18) peak at m/z 396. The compound was identified as β-sitosterol in conformity with an earlier paper (Shaban et al., 1988).

A pure sample of **5b** was oxidized to the corresponding ketone by using pyridinium chlorochromate (accompanied by the usual shifting of double bond to C-3 position), the NMR and mass spectra of which matched with that of **4b**, confirming the identification of both.

Table I. Analysis of Hydrocarbons of NSF of Cardamom

compd	mol formula	M+	major characteristic MS peaks (m/z)
saturated			
1a, *n*-tricosane[a]	$C_{23}H_{48}$	324	57, 71, 85, 99, 43, 127, 141, 155
1b, *n*-pentacosane	$C_{25}H_{52}$	352	
1c, *n*-heptacosane[a]	$C_{27}H_{56}$	380	
1d, *n*-nonacosane	$C_{29}H_{60}$	408	
1e, *n*-hentriacontane[a]	$C_{31}H_{64}$	436	
1f, *n*-tritriacontane	$C_{33}H_{68}$	464	
unsaturated			
1g, tricosene[a]	$C_{23}H_{46}$	322	57, 55, 71, 69, 85, 83, 99, 97
1h, pentacosene[a]	$C_{25}H_{50}$	350	
1i, heptacosene[a]	$C_{27}H_{54}$	378	
1j, nonacosene[a]	$C_{29}H_{58}$	406	
1k, hentriacontene[a]	$C_{31}H_{62}$	434	
1l, tritriacontene[a]	$C_{33}H_{66}$	462	

[a] Compounds newly reported.

Table II. Mass Spectral Data of Residual Volatile Compounds Isolated from NSF of Cardamom

compd	mol formula	M+	MS peaks (m/z) in the order of % rel intensity
fraction A			
2a, α-copaene	$C_{15}H_{24}$	204	105 (100), 93 (98), 67 (96), 133 (42), 161 (32), 204 (18)
2b, α-ylangene	$C_{15}H_{24}$	204	105 (100), 91 (94), 161 (82), 79 (68), 204 (14)
3a, nerolidol	$C_{15}H_{26}O$	222	69 (100), 81 (56), 95 (16), 137 (12), 207 (2), 222 (0)
6a, eugenyl acetate[a]	$C_{12}H_{14}O_3$	206	164 (100), 149 (20), 131 (12), 43 (11), 206 (4)
fraction B_2			
α-terpineol	$C_{10}H_{18}O$	154	59 (100), 93 (58), 81 (37), 21 (37), 136 (24), 154 (0)
bisabolene	$C_{15}H_{24}$	204	69 (100), 93 (68), 55 (38), 81 (32), 107 (26), 204 (2)
phytol[a] (tentative)	$C_{20}H_{40}O$	296	71 (100), 57 (58), 69 (56), 81 (52), 55 (48), 296 (2)

[a] Compounds newly reported.

Table III. Mass Spectral Data of Sterols in NSF of Cardamom

compd	mol formula	M+	MS peaks (m/z) in the order of % rel intensity
4b, β-sitostenone[a]	$C_{29}H_{48}O$	412	412 (100), 55 (70), 124 (62), 69 (45), 229 (32)
4c, unidentified		426	55 (100), 69 (97), 57 (95), 83 (64), 97 (52), 411 (6), 426 (3)
5a, stigmasterol	$C_{29}H_{48}O$	412	55 (100), 57 (80), 69 (79), 81 (72), 95 (60), 412 (50)
5b, β-sitosterol	$C_{29}H_{50}O$	414	414 (100), 55 (63), 107 (54), 81 (54), 396 (43), 303 (45)
5c, γ-sitosterol[a] (tentative)	$C_{29}H_{50}O$	414	95 (100), 93 (84), 71 (46), 145 (45), 55 (27), 83 (26), 414 (6)

[a] Compounds newly reported.

^1H NMR spectrum of the lowest R_f band (**5c**) showed resemblence to that of **5b** with a slight difference in the signal due to C_{29} methyl protons. The signals at δ 5.34 (1 H, d, C-4, olefinic proton) and multiplet at δ 3.51 (1 H) were similar. Signals at δ 0.68 (3 H, s, C-18), 0.80 (3 H, d, C-27, overlapped), 0.82 (3 H, d, C-26, overlapped), 0.84 (3 H, t, C-29), 0.92 (3 H, d, C-21), and 1.01 (3 H, s, C-19) accounted for six methyl groups. The slightly downfield signal due to C-29 when compared to the case of β-sitosterol was attributed to deshielding effect. The mass spectra of the compound showed base peak at m/z 95, M^+ at m/z 414, and a (M − 18) peak at m/z 396. The compound has been tentatively identified as γ-sitosterol.

Eugenyl Acetate. Fractions 24–28 afforded a brown resinous semisolid fraction. This on TLC (silica gel, hexane/EtOAc, 80:20) gave one clear spot, **6a** (R_f 0.70), along with a spot corresponding to β-sitosterol. NMR of the purified compound **6a** showed signals at δ 6.68 (1 H, d), 6.85 (1 H, d), and 6.95 (1 H, d) characteristic for aromatic protons. A strong signal at δ 5.49 (olefinic protons), a singlet at δ 3.84 (OCH_3), and another signal at δ 2.31 (acetate) suggested the compound to be eugenyl acetate, which remained undistilled and unreacted during the preparation of NSF of cardamom. MS of the compound [M^+ at m/z 206 and base peak at m/z 164 (M − CH_3CO)] confirmed this.

The analysis of fraction B_{1b} afforded all the compounds isolated from fractions 14–20/21–23 earlier. GC–MS analysis of the liquid fraction, B_2 (Table II), showed α-terpineol, bisabolene, nerolidol, and phytol as the major components, all except the last having been already reported in cardamom (Purseglove et al., 1981; Lawrence, 1979, 1989).

ACKNOWLEDGMENT

We thank Prof. F. Bohlmann and Prof. J. Jakupovic, Institute for Organic Chemistry, Technical University of Berlin, for providing necessary facilities and guidance to M.G. for carrying out the work in their institute. We also thank Dr. A. D. Damodaran, Director, RRL, Trivandrum, for his keen interest in the work.

LITERATURE CITED

Coscia, C. J.; Zweig, G.; Sherma, J., *CRC Handbook of Chromatography*; CRC Press: Boca Raton, FL, 1984; Vol. 1.

Fieser, L.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. I, p 61.

Grasselli, J. G.; Ritchey, W. M. *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds*; CRC Press: Cleveland, OH, 1975; Vol. I–IV.

Gunstone, F. D.; Harwood, J. L.; Padley, F. B. *The Lipid Handbook*; Chapman and Hall: London, 1986; p 477.

Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas-Chromatography*; Academic Press: New York, 1980.

Kasturi, T. R.; Iyer, B. H. Fixed Oil from Elettaria Cardamomum Seeds. *J. Indian Inst. Sci.* **1955**, *37A*, 106–112.

Kotaoka, E.; Tokue, C.; Tonimura, W. Findings on Sterol, Tocopherol and Phospholipids in Cardamom. *Nogaku Shuho* **1987**, *31* (3), 189–196 (Japanese).

Lawrence, B. M. *Essential Oils 1976–1978*; Allured Publishing: IL, 1979; pp 138–139.

Lawrence, B. M. Progress in Essential Oils. *Perfum. Flavor.* **1989**, *14* (6), 87–90.

Marsh, A. C.; Moss, M. K.; Murphy, E. W. Composition of Foods, Spices and Herbs—Raw, Processed, Prepared. In *Agriculture Handbook*, No. 8-2; USDA Agricultural Research Service: Washington, DC, 1977.

Miyazawa, M.; Kameoka, H. The Constitution of the Essential Oil and Nonvolatile Oil from Cardamom Seed. *J. Jpn. Oil Chem. Soc. (Yukagaku)*, **1975**, *24*, 22–26.

MSDC. *Eight peak index of mass spectra*; The Royal Society of Chemistry: London, 1983.

Pierce, A. E. *Silylation of Organic Compounds*; Pierce Chemical: Rockford, IL, 1968; pp 33–71.

Pouchert, C. J.; Campbell, J. R. *The Aldrich Library of NMR Spectra*; Aldrich Chemical: Milwaukee, WI, 1974.

Purseglove, J. W.; Brown, E. G.; Green, C. L.; Robbins, S. R. J. Cardamom, In *Spices*; Longman Group: London, 1981; Vol. 2.

Rubinstein, I.; Goad, L. J.; Clague, A. D. H.; Mulheirn, L. J. The 220 MHz NMR Spectra of Phytosterols. *Phytochemistry* **1976**, *15*, 195–200.

Salzer, J. J. *Fette, Seifen, Anstrichm.* **1975**, *77*, 446–450.

Shaban, M. A. E.; Kandeel, K. M.; Yacout, G. A.; Mehaseb, S. Gas Liquid Chromatographic Analysis of the Lipid Fraction of Elettaria Cardamomum Seeds. *Acta Aliment.* **1988**, *17* (2), 95–101.

Szymanski, H. A.; Yelin, R. E. *NMR Band Handbook*; IFI/Plenum Data Corp.: New York, 1968.

Received for review January 9, 1990. Revised manuscript received June 5, 1990. Accepted June 13, 1990.

Registry No. **1a**, 638-67-5; **1b**, 629-99-2; **1c**, 593-49-7; **1d**, 630-03-5; **1e**, 630-04-6; **1f**, 630-05-7; **1g**, 56924-46-0; **1h**, 30551-31-6; **1i**, 67537-80-8; **1j**, 77046-61-8; **1k**, 77046-64-1; **1l**, 85792-06-9; **2a**, 3856-25-5; **2b**, 14912-44-8; **3a**, 142-50-7; **4b**, 1058-61-3; **5a**, 83-48-7; **5b**, 83-46-5; **5c**, 83-47-6; **6a**, 93-28-7; α-terpineol, 98-55-5; bisabolene, 495-62-5; phytol, 150-86-7.