Indian Journal of Chemistry
Vol. 45B, June 2006, pp. 1567-1571

Note

Steroid and aliphatic esters from the seeds of *Nigella sativa*

B K Mehta*, Meenal Gupta & Manjul Verma

School of Studies in Chemistry, Vikram University,

Ujjain 456 010, India

E-mail: bkmehta11@yahoo.com

Received 4 January 2005; accepted (revised) 9 December 2005

Three novel compounds, one steroid and the other two aliphatic esters, have been isolated from the seeds of *Nigella sativa* and identified as ergosta-5,24 (28)-dien-2,3-*cis*-diol **1**, pentyl undecanoate **2**, methyloctadeca-14,16-dienoate **3**, on the basis of spectral and chemical analysis. The compound **2** exhibits significant activity against *Staphylococcus aureus* and *Shigella spp*, while moderate activity against *Escherichia coli* has been recorded. Rest of the compounds show poor activity against all the tested bacteria.

Keywords: *Nigella sativa,* Ranunculaceae, ergosta-5,24 (28)-dien-2,3-*cis*-diol, pentyl undecanoate, methyloctadeca-14,16-dienoate

IPC: Int.Cl.[8] C07C

Nigella sativa Linn. (Kalaungi) belongs to family Ranunculaceae, it is a spicy plant and is reported to possess antibacterial, bronchodilatory, hypertensive, antitumour and immuno-protecting activity[1]. Its seeds are commonly believed to have carminative, stimulatory and diaphoretic properties[2,3]. The seeds contain an alkaloid nigellidine[4], sterols like cholestrol, campesterol, stigmasterol, β-sitosterol, α-spinasterol[5], saponin[6], nigellone[7], nigellimine[8] and nigellicine[9]. Herein is reported isolation and identification of three compounds from the unsaponifiable matter of *n*-hexane extract of *Nigella sativa* (Seeds) *viz*, ergosta-5,24(28)-dien-2,3-*cis*-diol **1**, pentyl undecanoate **2** and methyloctadeca-14,16-dienoate **3**.

Results and Discussion

Compound **1** was isolated from the *n*-hexane extract of the plant by column chromatography over alumina grade III. The structure was established by spectroscopic and chemical means.

It gave positive Salkowski and Liebermann-Burchard test specific for Δ^5-sterols[10,11]. The mass spectrum of **1** showed the molecular ion peak at m/z 414 corresponding to the molecular formula $C_{28}H_{46}O_2$. IR spectrum showed strong absorption bands for hydroxyl group (3399 cm^{-1}), unsaturation (1627 cm^{-1}), isopropyl group (1379 cm^{-1}) and exomethylene (891 cm^{-1})[12-14].

^{1}H NMR spectrum showed sharp singlets at δ 0.68 and 1.01 each for three protons showing the presence of two angular methyl groups. A doublet at δ 0.82 (J=6.0 Hz) was assigned to methyl protons at C-21. Two doublets for six protons were assigned to isopropyl group at the end of the side chain at δ 0.92 and 0.85 (J=7.5 Hz)[15-19]. The exocyclic olefinic protons in the side chain resonated at δ 4.39 and 4.42 as two singlets[20] while a broad doublet at δ 5.35 (J=8.0 Hz) for one proton was assigned to the olefinic proton at C-6 in the ring which is usually observed in steroids having Δ^5 as in gorgosterol and brassicasterol[17-19,21]. Both the -OH protons resonated at δ 1.5 as an intense singlet. A ddd at δ 3.43 and a ddd at δ 3.82 were assigned to the protons present at C-3 (J_{aa} = 11.4 Hz, J_{ae} = 4.2 Hz) and C-2 (J_{ea}, J_{ee} =2.7 Hz) respectively[22].

^{13}C NMR spectrum showed peaks at δ 11.8 and 19.8 for two angular methyl groups in the molecule and a singlet at δ 18.9 due to the methyl carbon at C-21. The carbinolic carbon at C-2 and C-3 resonated at δ 73.4 and 67.5 respectively. The quaternary carbon at C-5 resonated at δ 143.8, while C-6 resonated at δ 117.8. The methylene carbon C-25 resonated at δ 117.0 while quaternary carbon C-24 resonated at δ 142.0. ^{1}H-^{1}H connectivities were confirmed by 2D COSY NMR spectra (**Table I**).

Mass spectrum indicated the molecular ion peak at m/z 414 suggesting its molecular formula $C_{28}H_{46}O_2$. The fragmentation pattern is characteristic of steroid as it showed peaks at m/z 399 (M-CH_3), m/z 381 [M-(CH_3+H_2O)], m/z 396 (M-H_2O), m/z 303 (M-111), m/z 273 [M-(side chain + H_2O)] and m/z 255 [M-(side chain + 2OH)].

A relatively abundant fragment at m/z 396 showed that a cyclic ether is formed due to removal of water (m/z 18) moiety from vicinal diol (2–OH). Acetylation of **1** with Ac_2O/pyridine afforded a diacetate indicating the presence of two –OH groups in the compound.

Table I — ^{1}H -^{1}H COSY connectivities

Proton (δ)	Correlated proton (δ)
C-1 (1.85)	3.82, 3.43 (C-2, C-3)
C-2 (3.82)	1.85, 3.43 (C-1, C-3)
C-3 (3.43)	3.82, 2.20 (C-2, C-4)
C-6 (5.35)	1.85 (C-7)
C-12 (1.42)	1.01, 0.82 (C-19, C-21)
C-15 (1.50)	1.25, 2.00 (C-14, C-16)
C-16 (2.00)	1.50, 1.25 (C-15, C-17)
C-21 (0.82)	2.35 (C-22)

It was oxidized by Jones reagent ($Cr^{VI}O_3$, H_2SO_4) and gave a diketo product indicating that both the –OH groups are secondary alcoholic groups.

Based on above evidence, the compound **1** was identified as ergosta-5,24(28)-dien-2,3 *cis*-diol and is being reported for the first time (**Scheme I**).

Compound **2**. Mass spectral analysis gave the molecular formula of **2** (m/z 256) as $C_{16}H_{32}O_2$.

IR spectrum showed strong absorption for ester group (1729 cm^{-1}) and indicated the long chain aliphatic nature (1096, 730-720 cm^{-1}) of the molecule. In its ^{1}H NMR spectrum a triplet at δ 0.90 (*J*=7.5 Hz) for six protons was due to the terminal methyl groups.

Two triplets at δ 4.18 and 2.35 (2H, *J*=6.0 Hz) were due to the methylene protons of -CH_2-O-CO- and -CH_2-CO-O- moieties. Rest of the methylenes resonated at δ 1.30 as a sharp singlet. A broad singlet at δ 1.60 was due to four methylene protons β- to the ester group.

In the ^{13}C NMR spectrum a peak at δ 180.0 corresponds to the carbon of the ester group. Peaks at δ 34.5 and 32.0 correspond to the methylene carbons α- and β- to the ester group respectively. The signals at δ 29.7-22.7 corresponds to the remaining methylene carbons. The peak at δ 14.2 corresponds to the end methyl carbons.

Mass spectrum showed molecular ion peak at m/z 256. The position of the ester group was confirmed from its mass fragmentation pattern. It showed a peak at m/z 129 due to the McLafferty rearrangement. A peak at m/z 115 was due to the α-cleavage of ester group. Other abundant fragments at m/z 227, 199, 185, 171, 152, 111, 97, 83, 73 and 60 were also inconsistent with the proposed structure.

Alkaline hydrolysis of compound **2**, yielded a mixture of alcohol and carboxylic acid, identified as pentanol and undecanoic acid respectively.

Thus on the basis of the above evidence the compound **2** was identified as pentyl undecanoic acid (**Scheme I**).

Compound **3**. Mass spectral analysis gave the molecular formula of **3** (m/z 294) as $C_{19}H_{34}O_2$.

IR spectrum showed absorption for ester group (1730 cm^{-1}), unsaturation (1651, 1627 cm^{-1}) and indicated the long chain aliphatic nature (1030, 730-720 cm^{-1}) of the molecule.

In its ^{1}H NMR spectrum a peak at δ 1.60 as doublet for three protons was due to terminal methyl group near an unsaturation while a singlet at δ 3.64 for three protons was due to carbomethoxy group. Methylene protons of -CH_2-CO-O- moiety resonated as triplet at δ 2.36. A multiplet at δ 1.56 was due to the methylene protons β to a double bond and ester group. A multiplet at δ 5.35(*J*=5.4 Hz) was due to the vinylic protons, having *cis* configuration. The remaining methylenes resonated at δ1.25 as an intense singlet.

The ^{13}C NMR spectrum showed the terminal methyl carbon at δ 19.9. The carbon of ester group resonated at δ 174.0. The methine carbons C-14 and C-17 resonated at δ 118.0 while C-15 and C-16 resonated at δ 122.0. The methylene carbon, α- and β- to ester group resonated at δ 34.5 and 32.0 respectively and rest of the methylene carbons resonated at δ 29.8.

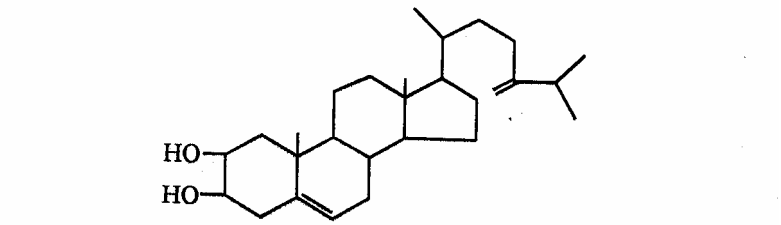
The base peak at m/z 81 in its mass spectrum was due to β-cleavage of the double bond. The abundant peak at m/z 235 was due to α-cleavage of the ester group. The abundant peaks at m/z 179, 164, 136, 109, 95, 74, 55 and 41 were in agreement with the proposed structure.

Compound **3** on alkaline hydrolysis gave a mixture of alcohol and carboxylic acid, identified as methanol and octadeca-14,16-dienoic acid respectively.

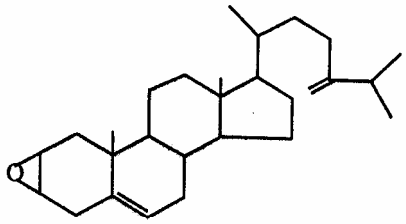
Thus, on the basis of above evidence the compound **3** was characterized as methyl octadeca-14,16-dienoate (**Scheme I**).

Screening of antimicrobial activity

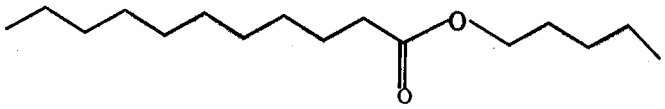
Agar diffusion technique was used for the screening of antimicrobial activity, using paper disc method[23,24]. The results are shown in **Table II**. The results have shown that compound **1** exhibited moderate to poor activity against *Staphylococcus aureus* and *Shigella spp*. Compound **2** showed significant activity against *Staphylococcus aureus* and *Shigella spp*., while moderate activity was displayed against *Klebsiella pneumoniae* and *Escherichia coli.*



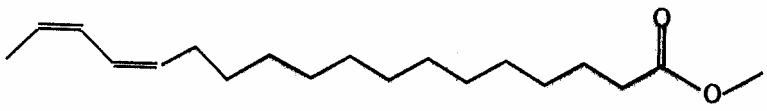
1



Cyclic ether of 1



2



3

Scheme I

Compound **3** exhibited moderate activity against *Staphylococcus aureus* and poor activity against *Shigella spp*. and *Klebsiella pneumoniae*. Unsaponifiable matter of *n*-hexane extract showed poor activity against *Proteus vulgaris* and *Klebsiella pneumoniae*.

Thus, only the compound **2** showed significant activity against *Staphylococcus aureus* and *Shigella spp*.

All the compounds exhibited nil or very low activity against all the tested fungi or yeast, while unsaponifiable matter of *n*-hexane extract showed significant activity against *Alternaria alternata*.

Experimental

Melting points are uncorrected. ^{1}H NMR was recorded on 300 MHz Varian XL spectrometer and 400 MHz Brucker WM spectrometer, ^{13}C NMR spectra were recorded on Varian XL 75 MHz spectrometer, IR spectra were recorded in KBr discs on Perkin-Elmer 377 spectrometer, EIMS were recorded on Jeol-JMS D 300 mass spectrometer. Column chromatography was carried out over alumina grade III and column grade silica gel, and TLC on silica gel G. Spots were visualized by iodine vapour or charring with H_2SO_4-vanillin spray. The seeds of *N. sativa* were collected from the nearby area of Ujjain city, and identified at the School of Studies in Botany, Vikram University, Ujjain.

Extraction and isolation of the compounds

The seeds (6 kg) were shade dried, cleaned, powdered and extracted with hexane in soxhlet extractor for 72 h. The extract was concentrated on a

Table II − Screening for antimicrobial activity

	Extract/Compounds			
Antibacterial activity	**1**	**2**	**3**	**4**
Staphylococcus aureus	++	+++	++	-
Proteus vulgaris	+	-	-	+
Escherichia coli	-	-	-	-
Shigella spp.	-	++	-	-
Citobacter fruendi	-	-	-	-
Klebsiella pneumoniae	-	+	-	+
Salmonella typhimurium	-	-	-	-
Antifungal activity				
Aspergillus niger	+	-	-	-
Aspergillus flavus	-	-	-	+
Penicillium notatum	-	+	-	-
Alternaria alternata	-	-	-	++
Candida albicans	-	-	+	-
Cunninghamella spp.	-	-	-	+

Disc diameter = 4.0 mm
- = No activity
+ = 6.8 - 8.0 mm
++ = 9.0 - 11mm
+++ = 12 - 14mm
++++ = 16 - 20mm
+++++ = 18 - 14mm
For antibacterial activity
The standards are in the form of sterile Hi-Disc cartridges, each disc containing 10 μg of the respective drug.
For antifungal activiy - Amphotericin B was used as standard.
1 ergosta-5,24(28)-dien-2,3 *cis*-diol, 2 pentyl undecanoate, 3 methyl octadeca-14,16-dienoate
4 unsaponifiable matter of *n*-hexane extract

rotary evaporator to afford 3500 mL oil. The oil was saponified by alcoholic potash method.Usual work-up yielded 116 g unsaponifiable matter, which was separated by repeated column chromatography on alumina grade III and silica gel, and monitored by TLC. The hexane:benzene (1:1 v/v) and methanol eluate yielded two compounds in pure form designated as compound **1** and **2** while hexane: benzene (1:3 v/v) eluate on rechromatography yielded compound **3** in pure form.

Compound 1. Ergosta-5, 24(28)-dien-2,3- *cis*-diol, (140mg, chloroform: methanol) m.p. 150-55°C M^+ 414; $C_{28}H_{46}O_2$, found: C, 80.9; H, 11.0.Calc. C, 81.1; H, 11.1%. Isolated from methanol fraction of the column. It showed a single homogenous spot on TLC using solvent system benzene: ether: acetic acid (8:2:1 v/v). IR (KBr): 3399, 2959, 2934, 2869, 1627, 1461, 1379, 1265, 1165, 1024, 730-720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.68 (3H, s, -CH_3, C-18), 1.01 (3H, s, -CH_3, C-19), 0.82 (3H, d, -CH_3, C-21, *J*=6.0 Hz), 0.92 (3H, d, -CH_3, C-26, *J*=7.5 Hz), 0.85 (3H, d, -CH_3, C-27, *J*=7.5 Hz), 4.39 (1H, s, C-28), 4.42 (1H, s, C-28), 5.35 (1H, br d, C-6, *J*=8.0 Hz), 1.5 (2H, s, 2×-OH, C-2 and C-3), 3.82 (1H, ddd, C-2, J=2.7Hz), 3.43 (1H, ddd, C-3, *J*=11.4, 4.2 Hz); EIMS: m/z (%) 414 [M^+] (61.0), 399 (21.1), 397 (33.7), 396 (30.9), 381 (12.4), 329 (17.9), 314 (9.1), 303 (17.3), 273 (15.1), 255 (19.9), 231 (12.9), 213 (19.8), 159 (17.6), 145 (25.3), 119 (17.8), 107 (25.6), 95 (30.5), 81 (34.1), 69 (36.8), 57 (58.2), 43 (100); ^{13}C NMR ($CDCl_3$): δ 39.5, 73.4, 67.5, 33.0, 117.8, 143.8, 37.3, 18.9, 19.8, 42.8, 11.8, 110.0, 121.0.

Acetylation of compound **1**: compound **1** (40 mg) was heated with Ac_2O (25 mL) and pyridine (10 mL) over a steam bath. The completion of the reaction was checked by TLC. The reaction mixture was worked up by usual method to afford eargosta-5,24 (28) -dien -2,3-*cis*-diacetate, m.p. 140-42°C.

Oxidation of compound **1**: compound **1** (80 mg) was dissolved in acetone (80 mL) and treated with Jones reagent (15 mL). The reaction mixture was stirred at RT and the progress was monitored by TLC. On completion of reaction, it was diluted with water and extracted with ether. Removal of solvent yielded a solid. Purification by recrystallization from ether:methanol gave the pure compound, m.p. 175°C.

Compound 2. Pentyl undecanoate, (25 mg, methanol) m.p. 103-04°C M^+ 256. $C_{16}H_{32}O_2$ found: C, 74.7; H,12.2. Calc. C, 74.9; H, 12.5 %. Isolated from hexane:benzene (1:1 v/v) fraction of the column. On TLC examination using solvent system hexane: ether:acetic acid (9:1:1 v/v) it gave a single homogenous spot. IR (KBr): 2920, 2851, 1729, 1705, 1462, 1298, 1183, 1096, 730-720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (6H, t, 2×-CH_3, *J*=7.5 Hz), 4.18 (2H, t, -CH_2-O-CO-, *J*=6.0 Hz), 2.36 (2H, t, -CH_2-CO-O-, *J*=6.0 Hz), 1.6 (4H, m, 2×-CH_2, β to ester group), 1.30 (18H, s, 9×-CH_2); EIMS m/z (%) 256 [M^+] (1.5), 239 (0.6), 227 (1.0), 213 (4.0), 199 (6.2), 185 (4.8), 171 (8.3), 152 (10.2), 143 (3.7), 129 (15.8), 111 (13.5), 97 (40.4), 83 (90.9), 73 (100), 60 (92.5), 55 (95.8);^{13}C NMR ($CDCl_3$): δ 180.0, 34.5, 32.0, 29.7, 29.4, 29.3, 29.2, 28.7, 26.0, 25.1, 22.7 and 14.2.

Compound 2 (2.0 mg) was refluxed with ethanolic KOH (1.3 mL, 5%) for 1 h. At the end of the reaction, the mixture was diluted with water (3.0 mL) and extracted with chloroform. The chloroform layer was dried over anhydrous magnesium sulphate and concentrated. To separate both the compounds the concentrated organic layer was put in deep freezer. After 4 days a semi solid mass separated out. After

the usual work-up, it was identified as acid (IR: 3420, 1720 cm^{-1}) and the liquid gave a positive test for alcohol.

Compound 3. Methyl octadeca-14, 16-dienoate, (30 mg, methanol) m.p. 82-3°C; M^+ 294. $C_{19}H_{34}O_2$, Found: C,77.2; H, 11.4. Calc. C, 77.5; H, 11.6 %. Isolated from hexane fraction of hexane:benzene (1:3, v/v) eluate of the column. It showed a single homogenous spot on TLC using hexane:ether:acetic acid (9.8:0.2:0.2, v/v) as solvent system. IR (KBr): 2958, 2919, 2854, 1730, 1651, 1627, 1170, 1020, 730-720 $cm^{-1;}$ ^{1}H NMR ($CDCl_3$): δ 1.60 (3H, d, -CH_3, *J*=7.5 Hz), 3.64 (3H, s, -CH_3), 2.36 (2H, t, -CH_2-CO-O-, *J*=6.0 Hz), 1.56 (4H, m, 2×-CH_2, β to double bond and ester group), 5.35 (4H, m, 2×-CH=CH-), 1.25 (18H, s, 9×-CH_2); EIMS: m/z (%) 294 [M^+] (5.5), 263 (4.8), 199 (2.2), 179 (3.6), 164 (20.0), 136 (19.2), 121 (26.1), 109 (24.8), 95 (66.1), 81 (96.2), 67 (99.7), 55 (100), 41 (70.0); ^{13}C NMR ($CDCl_3$): δ 174.0, 19.9, 118.0, 122.0, 34.5, 32.0, 29.8.

Compound 3 (2.5 mg) was refluxed with ethanolic KOH (1.5 mL, 5%) for 1 h. At the end of the reaction, the mixture was diluted with water (3.0 mL) and extracted with chloroform. The chloroform layer was dried over anhydrous magnesium sulphate and concentrated. To separate both the compounds the concentrated organic layer was put in deep freezer. After 4 days a solid separated out. After the usual work-up, the solid was identified as acid (IR: 3420, 1720, 1627cm^{-1}) and the liquid gave a positive test for alcohol.

Screening of antimicrobial activity

Agar diffusion technique was used for screening of antibacterial and antifungal activity using paper disk method[23,24]. The results are shown in the **Table II**.

Acknowledgement

Authors are grateful to RSIC, CDRI, Lucknow and RSIC, IIT Bombay, Mumbai for spectral analysis and UGC, New Delhi for financial assistance.

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