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PHYTOSTEROLS FROM *MURRAYA EXOTICA*

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Key Word Index—*Murraya exotica*; Rutaceae; leaves; novel phytosterols.

Abstract—From the cyclohexane extract of the leaves of *Murraya exotica*, five novel phytosterols: (23 *S*)-23-ethyl-24-methyl-cycloart-24(24[1])-en-3β-ol; 3β-methoxy-(23*S*)-23-ethyl-24-methyl-cycloart-24(24[1])-en-3β-ol; (23 *S*)-23-ethyl--24-methyl-cycloart-24(24[1])-3β-yl acetate; (23 ζ)-23-isopropyl-24-methyl-cycloart-25-en-3β-ol and (23 ζ)-23-isopropyl-24-methyl-cycloart-25-en-3β-yl acetate have been isolated. Structural elucidation of the isolated compounds is based on physical, chemical and spectral analysis including IR, ^{1}H and ^{13}C NMR and mass spectrometry.

INTRODUCTION

Our previous publications concerning phytosterols of the leaves of *Murraya exotica* revealed the isolation and identification of two new as well as two known phytosterols [1, 2]. As a continuation of our studies on *M. exotica*, the present paper describes the isolation and identification of a further five novel phytosterols from the leaves of the plant.

RESULTS AND DISCUSSION

The cyclohexane extract of *M. exotica* leaves on column chromatography gave two gummy materials. The first gummy mixture when chromatographed by HPLC using a reversed phase preparative RP-18 column gave compounds **1**, **2** and **4** while the second mixture yielded compounds **3** and **5** by argentation TLC.

Compounds **1–3** have the same '$C_{11}H_{21}$' side chain; this was deduced from mass spectral data (Table 1) of the isolated compounds, which showed significant peaks at *m/z* 297 [(M − ROH − SC (side chain)]$^+$ 407 (*k*-2), 315 (*e*) and 175 (*c*-SC) [3–5]. The presence of C-25, C-26 and C-27 as a terminal isopropyl unit is based on IR absorptions at 1385, 1375, 1180 and 1140 cm^{-1}; a ^{1}H NMR signal at δ1.15 (6H, *d*, *J* = 6.2 Hz) and ^{13}C NMR signals of C-25, C-26 and C-27 [6, 7]. The signals appearing at δ157.9 and 106.5 in the ^{13}C NMR spectra of compounds **1–3** confirmed the position of the exomethylenic double bond at C-24 [6, 7]. The methyl triplet at δ0.80 (*J* = 7.35 Hz) in the ^{1}H NMR spectrum and at δ13.0 in the ^{13}C NMR spectrum suggested the presence of an ethyl group at the C-23 position, probably having β-configuration [8–10]. The 9β,19-cyclopropyl structure of compounds **1–3** is based on the appearance of an IR absorption band characteristic for a cyclopropane methylene at 3042 cm^{-1}, AB quartets of *J* = 5 Hz in the ^{1}H NMR spectra (Table 2), the characteristic cleavage of 9–10, 5–6 and 9–19 bonds in the mass spectra with the formation of ion species-*c* [3–5] and ^{13}C NMR signals when compared with the previously isolated phytosterols [1–13].

Comparison of the ion species-*a* in the mass spectral data of the isolated compounds indicated that these compounds have OH (IR: 3432), OCH_3 (IR: 1100) and $OCOCH_3$ (IR: 1735 cm^{-1}) at position C-3 in compounds **1**, **2** and **3**, respectively. The α-orientation of H-3 was assigned on the basis of ^{1}H NMR signals appearing at δ3.42 (*t*, *J* = 5.2 Hz), 2.6–2.8 (*m*, *J* = 5 Hz) and 4.62 (*dd*, *J* = 5.3 and 11.0 Hz) in the ^{1}H NMR spectra of **1**, **2** and **3**, respectively [5, 11]. This conclusion was confirmed by close inspection of the ^{13}C NMR signal of C-3 in **1** and **3**, which appeared at δ77.9 and 79.7 (which is probably axial, the equatorial is downfield shifted by *ca* 3 ppm) [12, 13].

Based on the above data, compound **1** is assigned as (23 *S*)-23-ethyl-24-methyl-cycloart-24(24[1])-en-3β ol; compound **2** is 3 β-methoxy (23 *S*)-23-ethyl-24-methyl-cycloart-24(24[1])-en-3β ol and compound **3** is (23 *S*)-23-ethyl-24-methyl-cycloart-24(24')-en-3β-yl acetate. The occurrence of a phytosterol with the 9β,19-cyclotetracyclic structure having 33 carbon atoms in its skeleton and carrying an ethyl group at C-23 (**1**), together with its methyl ether (**2**) and its acetate (**3**), is reported here for the first time in nature.

Compounds **4** and **5** have the same $C_{12}H_{23}$ side chain as deduced from their mass spectral data which showed significant peaks at *m/z* 297 [M − ROH − SC]$^+$, 421 (*k*-2), 315 (*e*) and 175 (*c*-SC) [3–5]. The presence of C-25, C-26 and C-27 as an isopropenyl unit was based on IR absorptions at 1625 and 890 cm^{-1}; the characteristic ^{1}H NMR signals for C=CH_2, C=C–CH_3 (Table 2) and ^{13}C NMR signals for C-25, C-26 and C-27 [1, 7] (Table 3). The location of the exomethylenic double bond at C-25 was deduced from ^{13}C NMR signals at δ151.7 and

Table 1. Significant fragments in the mass spectra of compounds **1–5**

Compound	$[M]^+$	$[M-ROH]^+$	*a*	*b* = 1	*c*	*c*-SC	*d*	*e*	$[M-ROH-SC]^+$	*k*-2*
1	468	450	69	381	328	175	257	315	297	407
2	482	450	101	381	328	175	257	315	297	407
3	510	450	129	381	328	175	257	315	297	407
4	482	464	69	395	342	175	257	315	297	421
5	524	464	129	395	342	175	257	315	297	421

*This ion arises by loss of C_3H_7 from $[M-ROH]^+$, not as 41 but as 43 (Alpin and Horn by). [3, 5].

Table 2. ^1H NMR signals of compounds **1–5** ($CDCl_3$, 400 MHz)

Proton	**1**	**2**	**3**	**4**	**5**
3α-H	3.42 *t*	2.8 *m*	4.62 *dd*	3.45 *t*	4.61 *dd*
	J = 5.2	*J* = 5.0	*J* = 5.3, 11	*J* = 5.2	*J* = 5.3, 11
18-H3	0.95 *s*	0.96 *s*	0.95 *s*	0.95 *s*	0.95 *s*
19-H2	0.83 *d*	0.31 *d*	0.32 *d*	0.82 *d*	0.32 *d*
	0.55 *d*	0.54 *d*	0.55 *d*	0.54 *d*	0.57 *d*
	J = 5	*J* = 5	*J* = 5	*J* = 5	*J* = 5
21-H3	0.85 *d*	0.85 *d*	0.85 *d*	0.86 *d*	0.86 *d*
	J = 6.2	*J* = 6.2	*J* = 6.2	*J* = 6.2	*J* = 6.2
23^2-H3	0.80 *t*	0.80 *t*	0.80 *t*	0.91 *d*	0.91 *d*
	J = 7.35	*J* = 7.35	*J* = 7.35	*J* = 6	*J* = 6
23^2-H3	—	—	—	0.91, *d*	0.91, *d*
				J = 6	*J* = 6
24^1-H2	4.62 *bd*	4.61 *bd*	4.61 *bd*	—	—
	4.79 *bd*	4.80 *bd*	4.81 *bd*		
	J = 2.3	*J* = 2.3	*J* = 2.3		
24^1-H3	—	—	—	0.88 *d*	0.88 *d*
				J = 7.5	*J* = 7.5
26-H3	1.15 *d*	1.16 *d*	1.15 *d*	—	—
	J = 6.2	*J* = 6.2	*J* = 6.2		
26-H2	—	—	—	4.61 *bd*	4.61 *bd*
				4.81 *bd*	4.81 *bd*
				J = 2.2	*J* = 2.2
27-H3	1.15 *d*	1.16 *d*	1.15 *d*	1.60 *s*	1.60 *s*
	J = 6.2	*J* = 6.2	*J* = 6.2		
28-H3	0.97 *s*	0.92 *s*	0.89 *s*	0.97 *s*	0.85 *s*
29-H3	0.80 *s*	0.84 *s*	0.85 *s*	0.80 *s*	0.85 *s*
30-H3	0.88 *s*	0.88 *s*	0.88 *s*	0.88 *s*	0.88 *s*
OCH_3	—	3.34 *s*	—	—	—

Coupling constant (*J*) in Hz.

109.4 [7]. The presence of a methyl group at C-24 having the β-configuration was deduced from the appearance of a doublet at δ0.88 (*J* = 7.5 Hz) in the ^1H NMR spectra and at δ12.2 in the ^{13}C NMR spectra [7–10]. There is clear evidence for the presence of an isopropyl group in compounds **4** and **5** (IR absorptions at 1385, 1375, 1185 and 1140 cm^{-1}) [5] and its location at C-23 was deduced from the study of the mass spectral fragmentation pattern along the side chain. It was found that when the C-23/C-24 bond was cleaved, the isopropyl group remained in the major fragment (containing the ring system). This was confirmed by the fragment at *m/z* 395, but fission at C-22/C-23 led to the loss of an isopropyl group (i.e. the fragment at *m/z* 342 was observed) [5].

Comparison of the ion species-*a* in the mass spectral data for compounds **4** and **5** showed the presence of OH (IR: 3432) and CH_3CO (IR: 1735 cm^{-1}) at position C-3, respectively. The β-orientation of these groups was deduced from the ^1H and ^{13}C NMR spectral data (Tables 2 and 3) as described above.

Based on the above data, compound **4** is assigned the structure (23ξ)-23-isopropyl-24-methyl-cycloart-25-en-3β-ol and compound **5** as (23ξ)-23-isopropyl-24-methyl-cycloart-25-en-3β-yl acetate. The occurrence of a phytosterol alcohol and its acetate with the 9β,19-cyclotetracyclic structure, with 34 carbon atoms in its skeleton and carrying an isopropyl group at C-23, is reported here for the first time. The methyl ether of

Table 3. ^{13}C NMR signals of compounds **1** and **3–5** ($CDCl_3$)

Carbon no.	1	3	4	5
1	32.2	30.6	32.1	30.6
2	30.2	26.3	30.5	26.3†
3	77.9	79.7	78.0	79.9
4	40.5	38.7	40.5	38.7
5	48.1	48.8	48.2	48.8
6	20.7*	20.8*	20.7*	20.8*
7	28.0	28.0	28.0	28.0
8	47.9	48.1	47.8	48.1
9	20.1*	20.0*	20.2*	19.9*
10	25.8†	25.7†	25.8†	25.6†
11	25.6†	25.4†	25.7†	25.4†
12	35.8	36.1	35.8	36.2
13	45.3	45.2	45.3	45.2
14	48.7	48.9	48.7	48.9
15	33.0	33.0	33.0	32.9
16	26.3†	26.3†	26.3†	26.3†
17	52.2	52.2	52.2	52.3
18	18.0	18.0	18.0	18.0
19	29.9	29.8	29.9	29.9
20	35.9	35.8	36.1	35.6
21	18.3	18.4	18.3	18.4
22	30.5	30.4	23.0	23.0
23	49.2	49.3	56.4	56.6
23^1	27.4	27.4	28.0	28.1
23^2	13.0	13.1	21.1	21.0
23$^{2'}$	—	—	20.4	20.5
24	106.5	106.5	42.3	42.2
24^1	159.9	159.9	12.3	12.2
25	33.9	33.8	151.7	151.8
26	20.7*	20.8*	109.4	109.3
27	21.3	21.2	18.3	18.3
28	25.9†	26.0†	25.8†	26.1†
29	14.1	15.1	14.0	15.1
30	19.3	19.4	19.4	19.4
$\underline{C}OCH_3$	—	170.8	—	170.8
$CO\underline{C}H_3$	—	21.2	—	21.3

*, †Assignments may be interchangeable.

compound **4** has been isolated previously from *Skimmia wallichii* and given the trivial name skimmiwallichin [5].

EXPERIMENTAL

Mps are uncorr. Optical rotations were measured in $CHCl_3$. IR spectra were recorded in KBr with a Perkin Elmer instrument. ^{1}H NMR spectra were determined in $CDCl_3$ at 400 MHz with TMS as int. standard with a Brucker, Model GX 400. Mass spectra were recorded with a Finnigan 4000, EI spectrometer operating at 70 eV. ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as int. standard with a Brucker-Physik, Model WP 80. CC: alumina (E. Merck). TLC: Silica gel G (E. Merck) and $AgNO_3$ (20%) impregnated Silica gel G plates, 0.1 mm. HPLC: pump model 303, Gilson; UV detector: variable, Holochrome; injection system: Rheodyne; column, Lichrosorb RP-18.

Extraction and isolation. The leaves of *M. exotrica* L. were collected in October 1993 from plants growing in the Botanic Island in Aswan (Upper Egypt). The leaves were air-dried, ground to a fine powder and kept in a well-closed dark container. The conc. MeOH extract of 5 kg air-dried leaves was extracted with cyclohexane. The solvent free cyclohexane extract (70 g) was used. A portion of the extract (30 g) was subjected to CC on alumina using *n*-hexane–EtOAc (9:1). The phytosterols were present in two gummy frs with R_f 0.44 and 0.49 [silica gel TLC, *n*-hexane–EtOc (9:1)]. The gummy fr. with R_f 0.44 (350 mg) was examined for isolation of its components by the usual chromatographic methods, but these trials were unsuccessful. Therefore, it was subjected to HPLC on a reversed phase prep. BP-18 column, with MeOH–EtOH (3:1) as developer, flow rate 1.5 mg min^{-1}, UV detector operating at 215 nm, range 1.28. Compounds **1** (42 mg), **2** (11 mg) and **4** (70 mg) were isolated.

The other fr. (270 mg) was sepd into two bands by argentation TLC using CH_2Cl_2–CCl_4 (1:3). After purification by repeated argentation TLC and recrystallization, the two bands gave compounds **3** (37 mg) and **5** (55 mg).

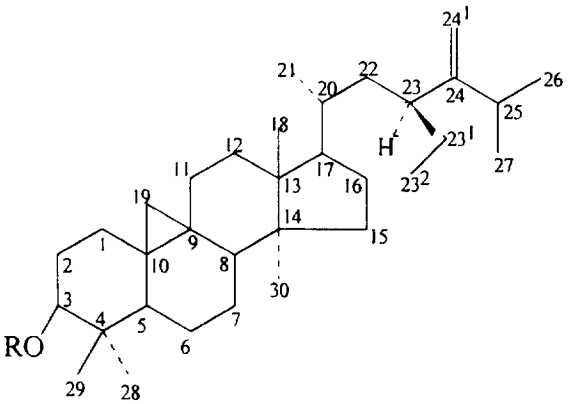
Compound **1**. Crystallized from MeOH as fine needles, mp 159–161°; $[\alpha]_D$ + 36° ($CHCl_3$); MS *m/z* (rel. int.); 468 $[M]^+$ ($C_{33}H_{56}O$) (21), 453 (16), 450 (7), 435 (3), 407 (2), 381 (3), 328 (10), 315 (11), 297 (10), 203 (26), 187 (30), 175 (67), 163 (47), 151 (23), 137 (22), 123 (43), 109 (62), 81 (56), 69 (77), 55 (100), 43 (35); IR γ_{max} cm^{-1}. 3432, 3042, 1385, 1375, 1180, 1140; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2).

Compound **2**. Crystallized from Me_2CO–$CHCl_3$ as fine needles, mp 153–154°; $[\alpha]_D$ + 33° ($CHCl_3$); MS *m/z* (rel. int.); 482 $[M]^+$ ($C_{34}H_{58}O$) (15), 467 (11), 450 (18), 435 (10), 407 (5), 381 (4), 328 (9), 315 (7), 297 (11), 203 (25), 187 (35), 175 (70), 163 (45), 151 (25), 137 (22), 123 (40), 109 (65), 101 (8), 81 (59), 69 (75), 55 (100), 43 (41); IR γ_{max} cm^{-1}: 3040, 1385, 1375, 1180, 1140, 1100, 980; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2).

Compound **3**. Crystallized from MeOH as fine needles, mp 151–152°; $[\alpha]_D$ + 62° ($CHCl_3$); MS *m/z* (rel. int.); 510 $[M]^+$ ($C_{35}H_{58}O_2$) (12), 495 (7), 450 (65), 435 (15), 407 (6), 381 (3), 328 (10), 315 (4), 297 (18), 203 (23), 187 (23), 175 (60), 163 (22), 151 (9), 137 (12), 129 (5), 123 (25), 109 (37), 81 (40), 69 (76), 55 (100), 43 (88); IR γ_{max} cm^{-1}: 3040, 1715, 1385, 1375, 1180, 1140, 980; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2).

Compound **4**. Crystallized from MeOH as fine needles, mp 155–157°; $[\alpha]_D$ + 41° ($CHCl_3$); MS *m/z* (rel. int.); 482 $[M]^+$ ($C_{34}H_{58}O$) (20), 467 (18), 464 (9), 449 (3), 421 (2), 395 (3), 342 (14), 315 (16), 297 (14), 203 (30), 187 (30), 175 (73), 163 (48), 151 (11), 137 (26), 123 (41), 109 (60), 95 (93), 81 (59), 69 (78), 55 (100), 43 (63); IR γ_{max} cm^{-1}: 3432, 3042, 1385, 1375, 1180, 1140, 980; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2).

Compound **5**. Crystallized from MeOH as fine needles, mp 148–149°; $[\alpha]_D$ + 52° ($CHCl_3$); MS *m/z* (rel. int.); 524 $[M]^+$ ($C_{36}H_{60}O_2$) (6), 509 (3), 464 (29), 449 (11), 421 (5), 395 (3), 342 (9), 315 (5), 297 (15), 203 (29), 187 (28), 175 (60), 163 (25), 151 (5), 137 (13), 123 (28), 109 (40), 95 (69), 81 (40), 69 (70), 55 (82), 43 (100); IR γ_{max} cm^{-1}: 3040, 1715,

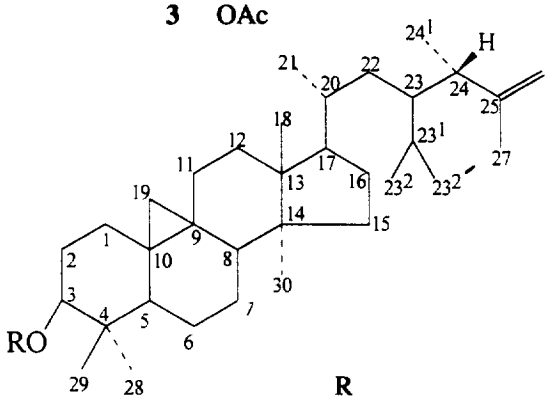


R

1 H

2 Me

3 OAc



R

4 H

5 OAc

1385, 1375, 1180, 1140, 980; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2).

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