Indian Journal of Chemistry
Vol. 44B, September 2005, pp. 1922-1926

Composition of a new chemotype of *Tanacetum nubigenum*

C S Chanotiya, S S Sammal & C S Mathela*

Department of Chemistry, Kumaun University, Nainital 263002, India

E-mail: mathelacs@rediffmail.com

Received 14 September 2004; (accepted) revised 5 January 2005

(3*R*,6*R*)-Tetrahydro-6-ethenyl-2,2,6-trimethyl-4*H*-pyran-3-acetate [3*R*,6*R*)-linalool oxide acetate] **1** (69.37%) along with 2-hydroxy-4,6-dimethoxyacetophenone **2**, *(E)*- and *(Z)*-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene **3** and **4**, ß-eudesmol **5** and selin-11-en-4α-ol **6** have been isolated from *Tanacetum nubigenum* Wall of Kumaon region of North Western Himalaya at an altitude of 3600-4300 m. (3*R*,6*R*)-linalool oxide acetate **1** has not been reported in *Tanacetum* species or from any other natural source. Presence of compounds **1-6** and absence of previously reported chrysanthenol and related esters makes it a new chemotype within the genus *Tanacetum*.

Keywords: *Tanacetum nubigenum*, Asteraceae, chemotype, (3*R*,6*R*)-Tetrahydro-6-ethenyl-2,2,6-trimethyl-4*H*-pyran-3-acetate, *(E)- & (Z)*-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene, 2-hydroxy-4,6-dimethoxyacetophenone.

IPC: Int.Cl.[7] A 61 K 31/00

The genus *Tanacetum*, commonly known as Tansy belongs to the family Asteraceae. Among the six species distributed in North Western Himalayan region, *T. nubigenum* Wall is an aromatic herb used as decoction to cure fever, dry material as incense by the local tribal inhabitants and grows in stony slopes and sandy grounds. It has taller stem (18-33 cm), smaller flower heads (3-5 mm across) and leaves, which are three times cut into linear acute lobe. It is a silvery tufted plant with usually many stems arising from the root stock[1,2].

The oil of *T. nubigenum* Wall was analyzed by GC and GC/MS. In addition to 10.7% of sabinene, nearly 44.0% of the oil of *T. nubigenum* Wall collected above 3600 m in the Kumaon region of Himalaya consisted of (-)-*cis*-chrysanthenol and its esters along with 3% of esters of *exo*-6-hydroxycamphor, 3,6;6,9-bisepoxyfarnesa-1,7(14),10-trien (1.6%), 6,9-epoxy-farnesa-1,7(14), 10-trien-3-ols (3.5%)[3].

Thus, the earlier reported constituents *viz*., (-)-*cis*-chrysanthenol and its esters were not noticed even as traces[3]. However, (3*R*,6*R*)-linalool oxide acetate, 2-hydroxy-4,6-dimethoxyacetophenone, *(E)*- and *(Z)*-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene, ß-eudesmol and selin-11-en-4α-ol were confirmed. Thus, it is a new chemotype of *T. nubigenum*.

Results and Discussion

From the essential oil of *T. nubigenum*, six compounds **1-6** were isolated with (3*R*,6*R*)-linalool oxide acetate (69.37%) as the major constituent.

The ^{13}C NMR and DEPT of compound **1** showed 12 signals, which could be attributed to four CH_3, three CH_2, two CH and three quaternary carbon atom. The ^{1}H and IR spectra showed the presence of an acetoxyl group (1742 cm^{-1}) with ^{13}C NMR resonance at δ 169.8. Two of the oxygen atoms were accounted for by an acetoxyl group and the remaining oxygen atom must be assigned to a pyran ring, which was verified by the presence of two low-field carbon atoms bearing oxygen (δ 73.8, 73.2). Having three degrees of unsaturation it would be monocyclic with one exocyclic double bond (δ 145.8, 110.4) and an acetoxyl carbon (δ 169.8) which was also established by HMBC experiment (**Figure 1**).

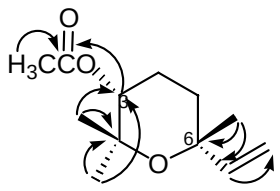
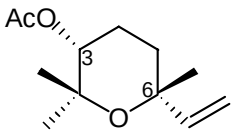
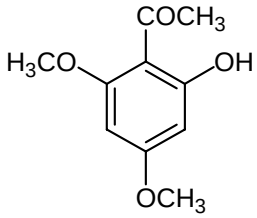


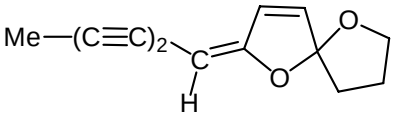
Figure 1 — ^{1}H-^{13}C long range coupling in **1**



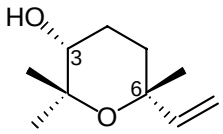
(3*R*,6*R*)-linalool oxide acetate **1**



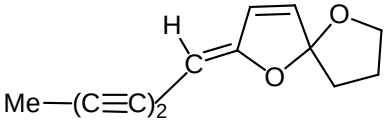
2-hydroxy-4,6-dimethoxy acetophenone 2



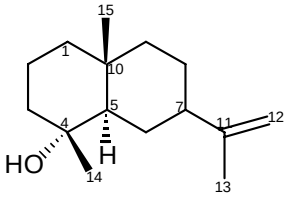
(*E*)-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene **3**



(3*R*,6*R*)-linalool oxide **7**



(Z)-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene **4**



Selin-11-en-4alpha-ol **6**

It is clear from ^{1}H, ^{13}C NMR and HMBC experiments that the compound possesses 2,2,6,6-tetrahydrosubstituted pyran ring, which is an important structural fragment of many oxygenated natural products and also constitutes the basic skeleton of the four diastereomeric pyranoid linalool oxides: (i) (3*R*, 6*R*), (ii) (3*S*, 6*R*), (iii) (3*R*, 6*S*) and (iv) (3*S*, 6*S*)[4,5] . The configuration of (3*R*,6*R*)-linalool oxide acetate was determined through comparison of ^{1}H and ^{13}C NMR data of the reduction product of **1** with those reported for the diastereomeric linalool oxides[5].

Compound **1** on reduction with $LiAlH_4$ in ether at 25^0C afforded compound **7** having the (M^+) m/z 170 in EIMS corresponding to molecular formula $C_{10}H_{18}O_2$.

The ^{13}C NMR and DEPT of the reduction product showed a total of 10 signals accounting for three CH_3, three CH_2, two CH and two quaternary carbons. The ^{1}H and ^{13}C NMR data for **7** are in agreement with literature report for linalool oxide basic structure. The configuration of **7** can be assigned as (3*R*,6*R*) because of total agreement of its ^{1}H and ^{13}C NMR data reported for (3*R*,6*R*) diastereomer which has biological importance as insect attractant in certain pollination systems[5].

The compound **2**, a white crystalline solid, was analyzed for $C_{10}H_{12}O_4$ (M^+, m/z 196). The ^{13}C NMR spectrum showed 9 signals for a total of 1°Carbons, which were assigned to 2 × OCH_3, one CH_3, two CH and five quaternary carbons by the DEPT spectra. The signal of carbonyl carbon was observed at δ 203.1. One methyl adjacent to a carbonyl group appeared as singlet at δ 2.61 in ^{1}H NMR and at δ 32.8 in ^{13}C NMR. A high intensity signal at δ 55.53 was assigned to two-methoxy groups and appeared at δ 3.82 (s, H-6), and 3.85 (s, H-4) in ^{1}H NMR. The two methine protons, (d, δ 6.05, *J*=3 Hz, H-3) and (d, δ 5.92, *J*=3 Hz, H-5) were assigned to *meta*-coupled protons of aromatic system. A singlet for one proton at δ 14.03 in ^{1}H NMR, a downfield D_2O exchangeable OH at position-2 is due to the OH (unsubstituted *para*-phenol system) proton chelated with carbonyl. Thus, this is an acetophenone derivative[6]. The OH group was also confirmed by IR spectrum (344°Cm^{-1}). Saxena *et. al.*[6] suggested it as the biological precursor of flavone, 5-hydroxy-7,4'-dimethoxyflavone.

The IR, ^{1}H and ^{13}C NMR data of the compounds **3** and **4** confirm these as (*E)* and (*Z)* isomers of 2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]non-3-ene[7-9]. The

Anthemideae tribe is especially rich in acetylenic metabolites[10] with stereomeric spiroketalenolethers as chemical markers of this tribe. Polyacetylenes play roles such as nematicidal, antibiotic, insect repellent etc. (*E*)-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,4]-non-3-ene has been reported to exhibit spasmolytic and antiphlogiastic properties[10].

The compound **5** has a molecular formula, $C_{15}H_{26}O$ (EIMS, M^+ at m/z 222). Its ^{1}H NMR showed signals at δ 0.70 (s, 3H) for angular methyl and a downfield signal at δ 1.20 (s, 6H, 2 × CH_3) for methyl attached to a hydroxyl group, two signals at δ 4.45 (s, 1H) and δ 4.71 (s, 1H) are for the methylene protons. The NMR assignments compare well with the literature report for ß-eudesmol[11-13].

Compound **6**, a crystalline solid, displayed a molecular ion peak at m/z 222 [M^+] in its EI-MS for $C_{15}H_{26}O$ and fragment peak at 204 due to the loss of H_2O suggesting the compound to be alcohol (IR: 330°cm^{-1}). Its ^{1}H NMR showed a total of three methyl signals at δ 0.89 (s, 3H) for angular methyl at δ 1.12 (s, 3H) attributed to the methyl attached with the carbon bearing hydroxyl group and a downfield signal at δ 1.75 (s, 3H) for methyl attached to exocyclic double bond, two proton multiplet signals at δ 4.70 (m, 2H) due to the exocyclic methylene protons. The ^{13}C NMR of the compound showed a total of 15 carbons and their multiplicity assignments marked the presence of three CH_3, seven CH_2, three CH and two quaternary carbon atoms. Compound **6** has thus been identified as selin-11-en-4α-ol as one of the eight stereoisomers of eudesm-11-en-4-ol[14-16].

Experimental Section

The 1D and 2D NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 MHz, ^{1}H and 75 MHz ^{13}C instrument using TMS as internal standard. The GC-MS was recorded on a ThermoQuest Trace GC 2000 interfaced with Finnigan MAT Polaris Q ion trap mass spectrometer using a RTX-5 MS non-polar capillary column (30m × 0.25 mm, 0.25 µm film coating), the oven temperature was programmed from 60°C at 3°C/ min ramp to 210°C of a final hold time of 10 min. The EIMS were recorded at 70 eV. GC was also carried out on Nucon 5765 Gas Chromatograph for quantitation using a RTX-5 MS non-polar capillary column (30m × 0.32 mm, 0.25 µm film coating). The CC was carried out on Merck 230-400 mesh silica gel and TLC on Merck silica gel-G.

Plant material

The aerial part of *T. nubigenum* was collected from Pindari glacier during Sept 2001 to Sept 2003. The voucher specimen No. Chem DST/ Tn: 01-05 were identified from Botanical Survey of India, Dehradun and Botany Department, Kumaun University and deposited in the Phytochemistry Lab., Department of Chemistry, Kumaun University.

Extraction and Isolation

The fresh aerial parts were steam distilled and separated in ether layer, which was dried over Na_2SO_4 followed by solvent removal with thin-film rotary evaporator. The residue/oil fractionated on silica gel CC with gradient elution from *n*-hexane to 20% Et_2O in *n*-hexane gave 6 compounds, which were purified by Waters' HPLC using µ-Porasil column (25 cm length × 7.8 mm), 2.0 mL/min flow rate, RI detector in an attenuation of 32× at 1000 Psi using 5.0-15.0 % Et_2O in *n*-hexane to give **1** (98.5 mg), **2** (20.0 mg), **3** (27.0 mg), **4** (33.8 mg), **5** (24.8 mg) and **6** (46.5 mg).

(3*R*,6*R*)-Linalool oxide acetate 1: Pale yellow liquid, $[\alpha]_D$ +29.32 ($CHCl_3$, c 0.32); IR (cm^{-1}): 1742; ^{1}H and ^{13}C NMR (see **Table I**); EIMS ($C_{12}H_{20}0_3$), 70 eV, m/z (rel. int.): 79 (100.00), 81 (10.00), 91 (23.29), 93 (73.93), 94 (39.83), 95 (22.80), 97 (10.43), 107 (14.96), 109 (15.77), 119 (15.39), 125 (09.72), 136 (12.00), 137 (46.79), 197 (15.65), 212 [M^+].

2-Hydroxy-4,6-dimethoxyacetophenone 2: White crystalline solid; IR (cm^{-1}): 3440; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.61 (3H, s, $COCH_3$), 3.82 (3H, s, OCH_3, H-6), 3.85 (3H, s, OCH_3, H-4), 5.92 (1H, d, *J*=1.8 Hz,

Table I — ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data in $CDCl_3$ for compound **1**

Position	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)
2	-	73.8
3	4.63 (1H, m)	75.7
4	2.13 (2H, m)	31.6
5	1.50-1.83 (2H, m)	21.9
6	-	73.2
7	5.96 (1H, AB*X*)	145.8
8	4.96-5.02 (2H, *AB*X)	110.4
9	1.16 (3H, s)	31.1
10	1.14 (3H, s)	21.6
11	1.20 (3H, s)	29.1
12	-	169.8
13	2.02 (3H, s)	20.8

H-5), 6.05 (1H, d, *J*=2.1 Hz, H-3), 14.03 (1H, s, D_2O exchangeable OH-2); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 32.8 (s, $COCH_3$), 55.5 (2 × OCH_3), 93.4 (d, C-3), 90.7 (d, C-5), 105.9 (s, C-1), 162.9 (s, C-2), 166.0 (s, C-6), 167.5 (s, C-4), 203 (C=O); EIMS ($C_{10}H_{12}O_4$), 70 eV, m/z (rel. int.): 65 (02.69), 93 (02.97), 95 (06.53), 109 (02.23), 111 (02.66), 121 (02.15), 123 (03.08), 125 (04.19), 137 (02.35), 138 (07.58), 151 (03.40), 153 (02.17), 163 (02.34), 166 (10.37), 178 (21.05), 179 (04.21), 181 (100.00), 182 (10.12), 196 [M^+] (28.98), 197 (03.31).

***(E)*-2-(2, 4-hexadiynylidene)-1,6-dioxaspiro[4,4]-non-3-ene 3**: Liquid; IR (cm^{-1}): 2230, 2125, 1630, 1580; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.98 (3H, s, H-1), 4.92 (1H, br s, H-6), 6.69 (1H, d, *J*= 5.7 Hz, H-8), 6.21 (1H, dd, *J*=5.7; 1.8 Hz, H-9), 2.30-2.00 (4H, m, H-11/12), 4.20-3.90 (2H, m, H-13); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 4.60 (q, C-1), 79.67 (s, C-2), 65.06 (s, C-3), 76.40 (s, C-4), 71.53 (s, C-5), 79.73 (d, C-6), 168.93 (s, C-7), 125.81 (d, C-8), 136.04 (d, C-9), 120.94 (s, C-10), 35.54 (t, C-11), 24.53 (t, C-12), 69.69 (t, C-13); EIMS ($C_{13}H_{12}O_2$), 70 eV, m/z (rel. int.): 76 (13.67), 102 (23.60), 115 (65.26), 116 (19.20), 127 (13.52), 128 (55.15), 129 (44.64), 141 (28.65), 143 (14.37), 153 (12.56), 157 (66.69), 158 (13.14), 169 (16.91), 170 (18.04), 171 (25.89), 172 (17.54), 185 (36.39), 199 (44.34), 200 [M^+] (100.00), 201 (14.16).

***(Z)*-2-(2, 4-hexadiynylidene)-1,6-dioxaspiro[4,4]-non-3-ene 4**: Liquid; IR (cm^{-1}): 2220, 2120, 1635, 1585; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.99 (3H, s, H-1), 4.59 (1H, br s, H-6), 6.22 (1H, d, *J*=5.7 Hz, H-8), 6.15 (1H, d, *J*= 5.6 Hz, H-9), 2.40-2.00 (4H, m, H-11/12), 4.30-3.90 (2H, m, H-13); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 4.74 (q, C-1), 80.58 (s, C-2), 65.40 (s, C-3), 79.94 (s, C-4), 70.77 (s, C-5), 78.83 (d, C-6), 167.16 (s, C-7), 127.45 (d, C-8), 135.25 (d, C-9), 121.06 (s, C-10), 35.61 (t, C-11), 24.48 (t, C-12), 69.66 (t, C-13); EIMS ($C_{13}H_{12}O_2$), 70 eV, m/z (rel. int.): 76 (13.19), 102 (23.71), 115 (64.66), 116 (19.40), 127 (14.85), 128 (52.53), 129 (43.52), 141 (27.88), 143 (14.25), 144 (13.51), 157 (61.87), 158 (15.41), 169 (18.23), 170 (19.00), 171 (27.11), 172 (15.17), 185 (36.84), 199 (43.92), 200 [M^+] (100.00), 201 (13.14).

ß-Eudesmol 5: Needle shaped crystals; IR (cm^{-1}): 3583, 3370, 3065, 1645, 1470, 1443, 1412, 1385, 1265, 1150, 1095; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (6H, s, H-12/13), 0.70 (3H, s, H-14), 4.45 (1H, s, H-15α), 4.71 (1H, s, H-15ß); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 41.1 (t, C-1), 23.3 (t, C-2), 36.9 (t, C-3), 151.1 (s, C-4), 49.8 (d, C-5), 24.9 (t, C-6), 49.4 (s, C-7), 22.4 (t, C-8), 41.8 (t, C-9), 35.9 (s, C-10), 72.9 (d, C-11), 26.8 (q, C-12), 27.1 (q, C-13), 16.3 (q, C-14), 105.3 (t, C-15); EIMS ($C_{15}H_{26}O$), 70 eV, m/z (rel. int.): 67 (29.08), 77 (19.57), 79 (36.99), 81 (29.94), 91 (35.24), 93 (48.90), 95 (16.75), 105 (34.79), 107 (34.70), 108 (44.56), 109 (16.97), 119 (19.01), 121 (26.53), 133 (24.90), 135 (17.54), 147 (16.04), 149 (100.00), 161 (23.82), 189 (25.67), 204 (17.21), 222 [M^+].

Selin-11-en-4α-ol 6: White solid crystals (CH_2Cl_2, c 0.5); IR (cm^{-1}): 3300; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, s, H-14), 1.12 (3H, s, H-15), 1.75 (3H, s, H-13), 1.81 (1H, dddd, H-5), 1.87 (1H, dquin, H-13), 1.96 (1H, dddd, br., H-7), 4.70, 4.72 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 43.3 (t, C-1), 20.1 (t, C-2), 44.6 (t, C-3), 72.2 (s, C-4), 54.9 (d, C-5), 26.0 (t, C-6), 46.3 (d, C-7), 26.8 (t, C-8), 41.0 (t, C-9), 34.6 (s, C-10), 150.7 (s, C-11), 108.1 (t, C-12), 22.7 (q, C-13), 21.0 (q, C-14), 18.6 (q, C-15); EIMS ($C_{15}H_{26}O$), 70 eV, m/z (rel. int): 67 (36.18), 79 (35.81), 81 (53.59), 91 (38.79), 93 (51.14), 95 (32.04), 105 (66.32), 107 (41.96), 119 (39.29), 121 (26.34), 133 (73.00), 134 (25.16), 135 (39.59), 147 (61.09), 148 (30.99), 161 (84.50), 162 (66.05), 175 (31.22), 189 (100.00), 204 (62.30), 222 [M^+].

(3*R*,6*R*)-Tetrahydro-6-ethenyl-2,2,6-trimethyl-4*H*-pyran-3-ol 7: White crystals; IR (cm^{-1}): 3426, 2975, 2945, 1634, 1450, 1407, 1362, 1229, 1185, 1153, 1114, 1085, 1001, 980, 909, 865, 830, 748; $[\alpha]_D$ + 1.9 (CH_2Cl_2, c 0.5); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (3H, s, H-9), 1.17 (3H, s, H-10), 1.25 (3H, s, H-11), 1.50-177 (2H, m, H-5), 2.13 (2H, m, H-4), 3.44 (1H, m, H-3), 4.95-5.01 (2H, *AB*X), 5.97 (1H, AB*X*); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 146.2 (d, C-7), 110.6 (t, C-8), 75.9 (s, C-2), 74.8 (d, C-3), 73.4 (s, C-6), 32.5 (t, C-4), 31.6 (q, C-9), 29.5 (q, C-11), 25.6 (t, C-5), 20.7 (q, C-10); EIMS ($C_{10}H_{18}O_2$), 70 eV, m/z (rel. int): 41 (16.00), 43 (17.51), 53 (09.82), 55 (09.26), 59 (24.83), 65 (17.26), 67 (94.02), 68 (10.10), 69 (06.65), 77 (22.26), 79 (100.00), 80 (07.89), 83 (06.87), 91 (07.79), 93 (07.09), 94 (23.58), 95 (09.89), 107 (07.13), 109 (07.72), 137 (06.51), 170 [M^+].

Acknowledgements

The authors are grateful to the DST, New Delhi for GC-MS grant and Fellowship to two of us (CSC and SSS), to BSI Dehradun, Botany Department, Kumaun

University, for plant identification and SAIF, CDRI, Lucknow, India for NMR and specific rotation measurements.

References

1 Polunin O & Stainton A, *Flowers of the Himalaya*, (Oxford University Press, New Delhi), **1984**, p.192.

2 Strachey R, *Catalogue of the plants of Kumaon and of the adjacent portions of Garhwal and Tibet*, (Periodical experts, Delhi, rep. edition), **1974**, p. 92.

3 Beauchamp P, Dev V, Kashyap T, Melkani A, Mathela C & Bottini A T, *J Essent Oil Res*, 13, **2001**, 319.

4 Tang R, Webster F × & Muller-Schwarze D, *J Chem Ecol*, 21, **1995**, 1745.

5 Vidari G, Rosa A D, Zanoni G & Bicchi C, *Tetrahedron: Assymmetry*, 10, **1999**, 3547.

6 Saxena S & Jain D C, *J Indian chem Soc*, 79, **2002**, 970.

7 Martinez V, Barbera O, Sanchez-Parareda J & Alberto Marco J, *Phytochemistry*, 26, **1987**, 2619.

8 Uchio Y, Tomosue K, Nakayama M, Yamamura A & Waki T, *Phytochemistry*, 20, **1981**, 2691.

9 Redaelli C, Formentini L & Santaniello E, *J Chromatogr*, 209, **1981**, 110.

10 Christensen L P, *Phytochemistry*, 31, **1992**, 7.

11 Cordano G, Merrien M A, Polonsky J, Rabanal R M & Varenne P, *J Indian chem Soc*, 55, **1978**, 1148.

12 Evans F E, Miller D W, Cairns T, Vernon B G & Wenkert E, *Phytochemistry*, 21, **1982**, 937.

13 Schwartz M A & Willbrand A M, *J Org Chem*, 50, **1985**, 1359.

14 Corbet R E & Smith R A J, *Tetrahedron Lett*, **1967**, 1009.

15 Weyerstahl P, Marschall-Weyerstahl H, Christiansen C, Oguntimein B O & Adeoye A O, *Planta Med*, **1988**, 546.

16 Kesselmans R P W, Wijnberg J B P A, Groot A D & Van Beek T A, *J Essent Oil Res*, **1992**, 4, 201.