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SESQUITERPENOIDS FROM *PALLENIS SPINOSA*

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Key Word Index—*Pallenis spinosa*; Compositae; sesquiterpenoids; germacranes; isodaucanes; pallenanes, cadinanes.

Abstract—The aerial parts of *Pallenis spinosa* gave a deoxygenated germacrane, an isodaucane ketal and the monoacetates of dihydroxypallenone and 3β-hydroxyoplopanone. © 1997 Elsevier Ltd

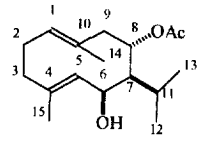
INTRODUCTION

Over the past few years, considerable attention has been given to *Pallenis spinosa* (L.) Cass. (family Compositae, tribe Inuleae, subtribe Inulineae), a monotypic genus widespread in the Mediterranean area [1]. The plant is a prolific producer of oxygenated sesquiterpenoids, including a ketone with a previously unreported carbon skeleton (dihyhdroxypallenone [2]). The presence of chemical races was also demonstrated. Thus, plant material collected in Sardinia, Egypt and Spain contained as major constituents germacrene D derivatives [3], oplopanones [2] and germacrene A-type epoxides [4], respectively. The plant has also been reported to cumulate 5-*O*-glycosyl flavones [5].

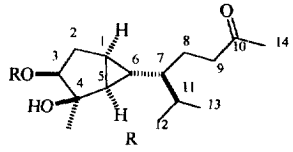
We have now investigated a new collection of *P. spinosa* from Sardinia. The aim was to better evaluate the infraspecific variation of the secondary metabolites within this species, and to shed light on its relationship with closely related genera (*Asteriscus*, *Buphtalmum*).

RESULTS AND DISCUSSION

Besides known compounds, four new sesquiterpenoids were isolated (**1–4**). The NMR spectra of **1** ($C_{17}H_{28}O_3$, MS) suggested a $\Delta^{1(10)}$, Δ^4-germacradiene structure with oxygen substituents at the carbons adjacent to the one bearing the isopropyl side chain. The chemical shift- and coupling constant pattern was similar to that of tovarol diacetate [6], with the notable exception of an upfield shift for H-6 ($\Delta\delta = -1.16$).



1



R
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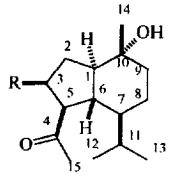
2b H



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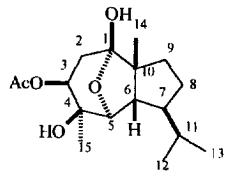
3b OH
3c H



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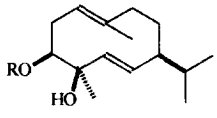
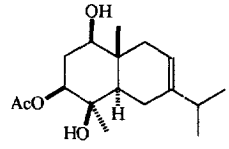


R
5a Ac
5b H



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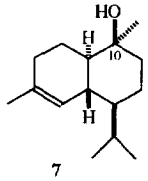

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*Authors to whom correspondence should be addressed.

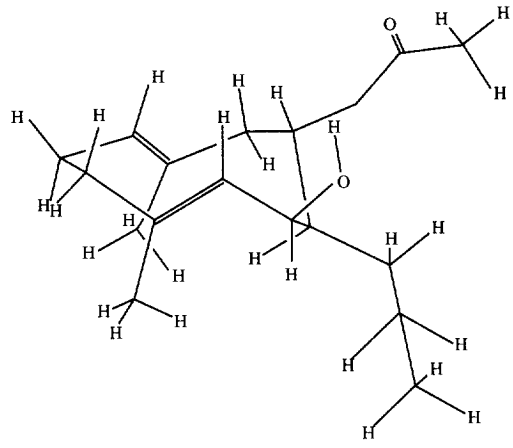

Fig. 1. Calculated conformation (PC model) of compound **1**.

This suggested that **1** is tovarol 8-acetate, as confirmed by comparison with the NMR data published for other tovarol 8-monoesters [7].* The values of $J_{6,7}$ (2.5 Hz) and $J_{7,8}$ (2.0 Hz) were very diagnostic, as was the detection of a NOE effect between H-6 and H-7. The NOE-effect between the allyl methyls (C-14 and C-15) showed that the major rotamer in solution has a conformation of the [$^1D_{14}$, $^5D_{15}$]-type [8]. Figure 1 depicts the calculated conformation (PC model) of **1**, which fits nicely the observed *J*-values and NOE interactions (H-15/H-7; H-8/H-1; H-14/H-15; H-6/H-7). The NOE-difference spectrum of **1** indicated also the existence of an additional conformation, which could not be further characterized because of its low concentration.

Compounds **2a** and **3a** were the monoacetates of two compounds previously isolated from an Egyptian collection of the plant [dihydroxypallenone (**2b**) and 3β-hydroxyoplopanone (**3b**)] [2], which were also present in our extract. Comparison of the ^{1}H NMR spectra of the acetates **2a**, **3a** and their corresponding alcohols **2b**, **3b**, showed the expected downfield shift of H-3 ($\Delta\delta + 1.04$ and $+0.88$, respectively). The NMR spectra of **2a** are identical to those of a compound reported as 3α-acetoxyoplopanone [9], whose structure should be revised.

The molecular formula of **4** ($C_{17}H_{28}O_5$, MS) and its NMR spectra showed the presence of a terpenoid core having three degrees of unsaturation and an acetoxy group. Since the ^{13}C NMR spectrum lacked olefinic carbons, compound **4** was tricyclic. The ^{1}H NMR spectrum disclosed the presence of an isopropyl group (δ 1.10 and δ 0.95, *d*, $J = 6.5$ Hz), a quaternary methyl (δ 1.21, *s*) and a methyl bound to an oxygenated quaternary carbon (δ 1.37, *s*). The ^{13}C NMR spectrum showed four oxygenated carbons, one of which was acetalic (δ 105.2, *s*). Since two of the oxygens had already been accounted for by the acetyl group, compound **4** had an oxygen bridge. The overlapping of the proton signals in the aliphatic region prevented the proton coupling network from being traced out in $CDCl_3$. However, the spectrum was better resolved in C_6D_6, and COSY experiments in this solvent established two spin sequences. One started from an alkoxymethine signal (H-5) at δ 3.47, and proceeded with two additional aliphatic methines (H-6 and H-7, δ 2.34 and 1.47, respectively), the most upfield of which (H-7) was further coupled to a $-CH_2-CH_2-R$ system and to the isopropyl group. The second spin system was an acyloxymethine (H-3, δ 4.89) coupled to a methylene. The detection of a *W*-coupling (*ca* 1.5 Hz) between the acetoxymethine- (H-3) and the alkoxymethine protons (H-5) correlated the two sequences, pointing to an isodaucane structure [10, 11]. The H-3/H-5 W-coupling and a near zero value for $J_{5,6}$ suggested a coplanar relationship between H-3 and H-5, and an orthogonal relationship between H-5 and H-6, respectively. Assuming the usual α-orientation for H-7, these *J*-values and the pattern of NOE effects (H-14/H-6; H-3/H-15) established the stereochemistry as depicted in **4**. The germacrene D derivatives **5a,b** [3], the eudesmane **6** [2] the oplopanone **3c** [2], the cadinane T-cadinol (**7**) [12] and spathulenol [13] were also isolated. Interestingly, the stereochemistry at C-10 of the cadinane **7** is opposite to that of the precursor of **2**–**4**.

Our results further substantiate *P. spinosa* as a rich source of oxygenated sesquiterpenoids. Infraspecific differences seemingly reflect the relative abundance of germacrene A- or germacrene D-derived compounds, and the extent to which germacrene D derivatives are cyclised to cadinanes and further fragmented. Indeed, the most remarkable feature of the species is the capacity to effect a pinacol-type fragmentation of cadinane glycols to isodaucane, pallenane, and oplopane derivatives, as summarised Fig. 2. Humulanolides (asteriscunolides) were detected in three species of *Asteriscus* (*A. aquaticus* L. [14], *A. graveolens* Less. [15], *A. sericeus* (L.f.) DC. [16]) recently transferred to the genus *Nauplius* [17, 18]. Asteriscunolides were not detected in the collections of *P. spinosa* investigated to date, nor were they in *A. pygmaeus* (DC.) Coss. et Desv. [19]. In the absence of a larger database of constituents for the genus *Asteriscus*, it is thus difficult to assess if the sesquiterpenoid pattern of *P. spinosa* supports the retaining of this monotypic genus, or if the plant should instead be transferred to *Asteriscus*, as recently suggested [17, 18].

*The *J*-values of H-8 and H-9b reported for the tovarol esters from *Ferula orientalis* L. var. *orientalis* (Miski, M., Mabry, T. J. and Saya, Ö. (1987) *Journal of Natural Products* **50**, 829) are a misprint (personal communication of Dr M. Miski).

EXPERIMENTAL

CC: silica gel Merck 70–230 mesh; HPLC: Waters microporasil column (0.8 × 30 cm), detection by a Waters differential refractometer 3401.

Plant material. Aerial parts of *P. spinosa* were col-

Table 1. ^{1}H NMR spectral data of compounds **1**, **2a**, **3a**, **3b** and **4** (400 MHz, $C\underline{H}Cl_3$ at δ 7.27 as reference, J in Hz)

H	**1**	**2a**	**3a**	**3b***	**4†**
1	5.00 *br d* (12.0)	1.04 *br ddd* (6.5, 5.0, 4.0)	1.42 *m*	1.39 *m*	—
2a	2.33 *m*	2.24 *ddd* (15.0, 7.0, 5.0)	2.33 *m*	2.31 *m*	2.10 *dd* (15.0, 1.0)
2b	2.12 *m*	1.81 *br d* (15.0)	1.42 *m*	1.30 *m*	1.94 *dd* (15.0, 6.5)
3a	2.12 *m*	4.65 *br d* (7.0)	5.35 *ddd* (9.5, 7.5, 7.0)	4.47 *ddd* (9.5, 7.5, 7.0)	4.89 *ddd* (6.5, 1.5, 1.5)
3b	2.12 *m*	—	—	—	—
4	—	—	—	—	—
5	5.18 *br d* (8.0)	1.16 *ddd* (6.5, 3.5, 1.0)	2.93 *dd* (10.0, 9.5)	2.93 *dd* (10.0, 9.5)	3.47 *br s*
6	4.44 *br dd* (8.0, 2.5)	0.89 *m*	2.04 *ddd* (10.0, 10.0, 10.0)	2.01 *ddd* (10.0, 10.0, 10.0)	2.34 *br d* (5.5)
7	1.20 *ddd* (11.0, 2.5, 2.0)	0.43 *dddd* (10.0, 8.0, 5.5, 4.0)	1.03 *m*	1.03 *m*	1.47 *m*
8a	5.10 *ddd* (12.0, 6.0, 2.0)	1.66 *m*	1.57 *m*	1.57 *m*	1.73 *m*
8b	—	1.57 *m*	1.09 *m*	1.08 *m*	1.25 *m*
9a	2.55 *br dd* (13.5, 6.0)	2.50 *br ddd* (16.5, 9.5, 5.5)	1.78 *m*	1.78 *m*	1.86 *m*
9b	2.00 *dd* (13.5, 12.0)	2.42 *br ddd* (16.5, 9.5, 5.5)	1.35 *m*	1.30 *m*	1.25 *m*
11	1.60 *dqq* (11.0, 7.0, 7.0)	1.75 *dqq* (7.0, 7.0, 4.0)	1.30 *m*	1.30 *m*	1.47 *m*
12	1.05 *d* (7.0)	0.93 *d* (7.0)	0.86 *d* (7.0)	0.87 *d* (7.0)	1.10 *d* (6.0)
13	1.03 *d* 7.0)	0.91 *d* (7.0)	0.66 *d* (7.0)	0.66 *d* (7.0)	0.95 *d* (6.5)
14	1.64 *br s*	2.14 *s*	1.19 *s*	1.19 *s*	1.21 *s*
15	1.42 *br s*	1.36 *s*	2.14 *s*	2.23 *s*	1.37 *s*
OAc	2.07 *s*	2.06 *s*	1.97 *s*	—	1.65 *s*

* Assignments reported in ref. [2] should be corrected.

† C_6D_6.

Table 2. ^{13}C NMR spectral data of compounds **1**, **2a**, **3a**, **3b**, **4** and **7** (75 MHz, $CDCl_3$, at 77.0 as reference)

C	1	2a	3a	3b*	4	7†
1	131.9 *d*	21.1 *d*	52.5 *d*	52.3 *d*	105.2 *s*	47.9 *d*
2	24.6 *t*	33.2 *t*	33.0 *t*	37.1 *t*	35.5 *t*	22.6 *t*
3	38.7 *t*	78.4 *d*	73.6 *d*	72.7 *d*	73.5 *d*	30.9 *t*
4	129.3 *s*	79.7 *s*	206.4 *s*	210.3 *s*	65.5 *s*	134.3 *s*
5	133.2 *d*	34.5 *d*	58.5 *d*	60.3 *d*	86.9 *d*	122.6 *d*
6	67.4 *d*	23.5 *d*	45.9 *d*	46.3 *d*	51.3 *d*	37.7 *d*
7	54.4 *d*	46.6 *d*	49.1 *d*	49.1 *d*	58.2 *d*	46.6 *d*
8	74.7 *d*	25.1 *t*	22.7 *t*	22.6 *t*	30.2 *t*	19.8 *t*
9	42.0 *t*	42.0 *t*	41.7 *t*	41.7 *t*	36.0 *t*	40.3 *t*
10	133.1 *s*	209.4 *s*	72.3 *s*	72.5 *s*	55.1 *s*	70.7 *s*
11	26.6 *d*	31.1 *d*	29.5 *d*	29.7 *d*	33.7 *d*	26.1 *d*
12	23.3 *q*	19.4 *q*	21.9 *q*	21.9 *q*	21.3 *q*	21.4 *q*
13	21.4 *q*	19.5 *q*	15.4 *q*	15.4 *q*	21.3 *q*	15.2 *q*
14	21.1 *q*	29.9 *q*	20.2 *q*	20.3 *q*	20.2 *q*	28.4 *q*
15	16.1 *q*	27.6 *q*	31.7 *q*	32.6 *q*	27.0 *q*	23.7 *q*
OAc	172.7 *s*	170.3 *s*	170.6 *s*	—	170.9 *s*	—
	21.2 *q*	21.3 *q*	21.0 *q*	—	21.2 *q*	—

* Assignments reported in ref. [2] should be corrected.
† Data not available in the literature.

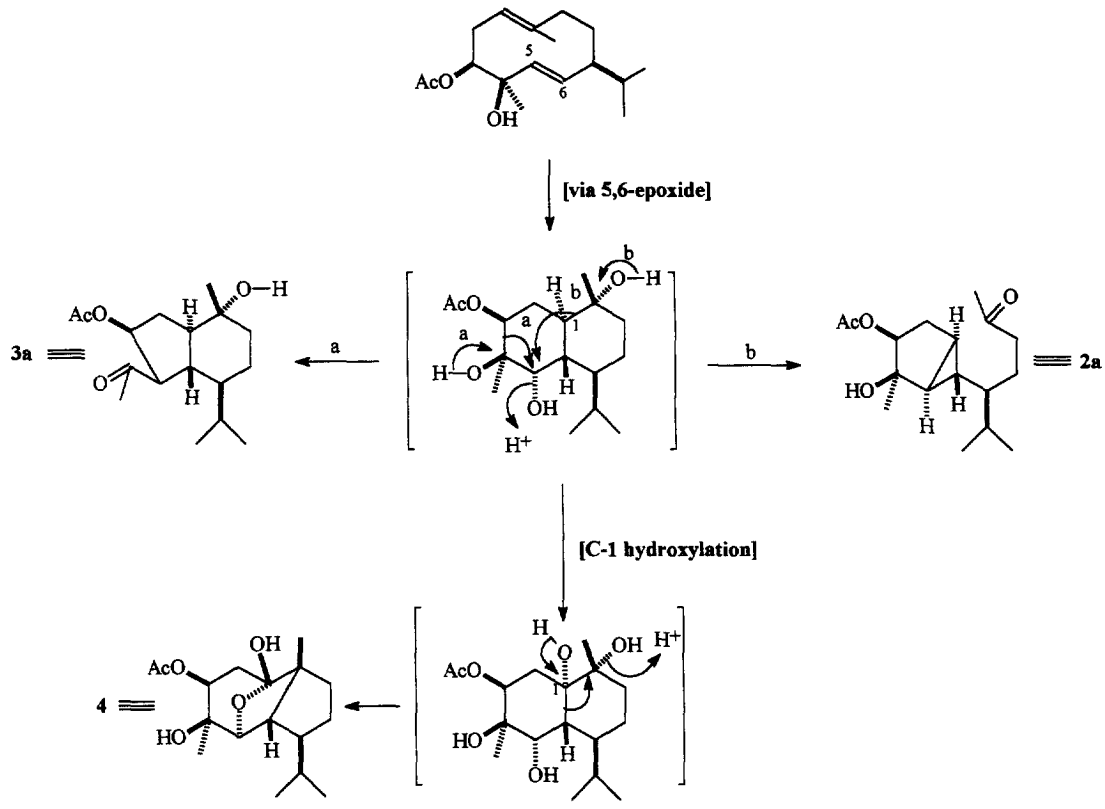

Fig. 2. Biogenetic relationship between the constituents of *P. spinosa*.

lected near Orosei (NU) in June 1992. The plant material was identified by V. Picci (University of Sassari). A voucher specimen is kept at the laboratory of Torino. Leaves and flowers showed the same TLC profile (hexane–EtOAc 5:5), and were pooled together.

Isolation of the constituents. Powdered non-woody aerial parts (leaves and flowers) were extracted with Me_2CO (3 × 1 l) at room temp. The extract was evapd and dissolved in EtOH (500 ml), and an aq./ soln of $Pb(OAc)_2$. $3H_2O$ (3%, 500 ml) was added. AFter 2 hr, the soln was filtered on Celite® and the filtrate was concd to *ca* 20 ml, diluted with water (300 ml) and extracted with EtOAc. Removal of the solvent left a

yellowish gum (4.0 g), which was sepd by CC [30 g silica, hexane–EtOAc gradient (from hexane–EtOAc 3:7 to pure EtOAc)]. According to differences in composition indicated by TLC, four crude frs (PS1–PS4) were obtained (705, 550, 615 and 200 mg, respectively). Fr. PS1 was further purified by CC (silica gel, hexane–EtOAc 19:1 as eluant) to give 23 mg **3c**, 37 mg **8** and 227 mg **5a**. Frs PS-2 and PS-3 were further sepd by CC (hexane–EtOAc 9:1 and 7:3, respectively) to give 68 mg **1**, 65 mg **5** (from PS-2), and 66 mg **6**, 60 mg **3a**, and impure **2a**, **4** and **3c** from PS-2. Further purification by HPLC (hexane–EtOAc 5:5) gave 33 mg, 27 mg and 35 mg pure **2a**, **4** and **3c**, respectively. Fr. PS-4 was further purified by CC (hexane–EtOAc 7:3) to give 10 mg **3b** and 13 mg **2b**.

8-Acetyltovarol (**1**). Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3400 (OH), 1735, 1240 (OAc). MS 140 eV, *m/z* (rel. int.): 280 $[C_{17}H_{28}O_3]^+[M]^+$ (2), 220 $[M\text{-}AcOH]^+$ (56), 205 $[M\text{-}Me]^+$ (20), 177 $[220\text{-}Me_2CH]^+$ (82), 159 $[177\text{-}H_2O]^+$ (70), 136 (85), 109 (52), 93 (75), 81 (100), 43 (99).

3β-Acetoxy-4β-hydroxypallenone (**2a**). Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3450 (OH), 1740, 1240 (OAc), 1705 (cyclopentanone); MS 140 eV, *m/z* (rel. int.): 296 $[C_{17}H_{28}O_4]^+[M]^+$ (3), 281, $[M\text{-}Me]^+$ (2), 236 $[M\text{-}AcOH]^+$ (16), 218 $[236\text{-}H_2O]^+$ (20), 193 $[236\text{-}Me_2CH]^+$ (40), 175 $[193\text{-}H_2O]^+$ (38), 123 (72), 109 (84), 97 (62), 81 (35), 43 (100).

3β-Acetoxyoplopanone (**3a**). Colourless oil. IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3450 (OH), 1735, 1250 (OAc), 1705 (ketone); MS 140 eV, *m/z* (rel. int.): 296 $[C_{17}H_{28}O_4]^+[M]^+$ (10), 281 $[M\text{-}Me]^+$ (8), 252 $[M\text{-}AcOH]^+$ (16), 218 $[236\text{-}H_2O]^+$ (20), 193 $[236\text{-}Me_2CH]^+$ (40), 175 $[193\text{-}H_2O]^+$ (38), 123 (72), 109 (84), 97 (62), 81 (85), 43 (100).

1α,5α-Epoxy-3β-acetoxy-6βH-isodaucane-1β,4β-diol (**4**). Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3450 (OH), 1730, 1240 (OAc); MS 140 eV, *m/z* (rel. int.): 312 $[C_{17}H_{28}O_5]^+[M]^+$ (1), 252 $[M\text{-}AcOH]^+$ (30), 234 $[252\text{-}H_2O]^+$ (10), 181 (100), 163 (36), 135 (60), 123 (80), 109 (42), 81 (68), 43 (78).

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REFERENCES

1. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A., *Flora Europaea*, Vol. 4. Cambridge University Press, London, 1976, p. 139.
2. Ahmed, A. A, Jakupovic, J. and Bohlmann, F., *Phytochemistry*, 1990, **29**, 3355.
3. Appendino, G., Cravotto, G., Gariboldi, P., Claudi, F. and Picci, V., *Phytochemistry*, 1989, **28**, 849.
4. Sanz, J. F. and Marco, J. A., *Phytochemistry*, 1991, **30**, 2788.
5. Ahmed, A. A., Spaller, M. and Mabry, T. J., *Biochemical Systematic and Ecology*, 1992, **20**, 785.
6. De Pasqual Teresa, J., Morán, J. R., Hernandéz, J. M. and Grande, M., *Phytochemistry*, 1985, **24**, 1779.
7. Marco, J. A., Sanz, J. F., García-Sarrión, A. and Rustaiyan, A., *Phytochemistry*, 1991, **30**, 2325.
8. Samek, Z. and Harmatha, J., *Collection of Czechoslovak Chemical Communications*, 1978, **43**, 2779.
9. Dupré, S., Grenz, M., Jakupovic, J., Bohlmann, F. and Niemeyer, H. M., *Phytochemistry*, 1991, **30**, 1211.
10. Yoshida, T., Muraki, S., Takahashi, K., Kato, T., Kabuto, C., Suzuki, T., Uyehara, T. and Ohnuma, T., *Journal of the Chemical Society, Chemical Communications*, 1979, 512.
11. Misra, L. N., Jakupovic, J., Bohlmann, F. and Schmeda-Hirschmann, G., *Tetrahedron*, 1985, **41**, 5353.
12. Cheng, Y. S., Kuo, Y. H. and Lin, Y. T., *Journal of the Chemical Society, Chemical Communications*, 1967, 565.
13. Bowyer, R. C. and Jefferies, P. R., *Chemistry and Industry*, 1963, 1245.
14. San-Feliciano, A., Barrero, A. F., Medarde, M., del Corral, J. M. M., Ledesma, E. and Sanchez-Fernando, F., *Tetrahedron Letters*, 1982, **23**, 3097.
15. El-Dahmy, S., Jakupovic, J., Bohlmann, F. and Sarg, T. M., *Tetrahedron*, 1985, **41**, 309.
16. Jakupovic, J., Lehmann, L., Bohlmann, F. and Arroyo Hogdson, A., *Phytochemistry*, 1987, **26**, 2854.
17. Wiklund, A., *Nordic Journal of Botany*, 1985, **5**, 299.
18. Wilkund, A., *Nordic Journal of Botany*, 1985, **7**, 1.
19. Ahmed, A. A., *Journal of Natural Products*, 1992, **55**, 824.