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NON-ACETALIC THAPSANE SESQUITERPENOIDS FROM *THAPSIA VILLOSA* VAR. *MINOR*

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Key Word Index—*Thapsia villosa* var. *minor*; Umbelliferae; sesquiterpenes; 6(14)-thapsene and 6,14-epoxythapsane esters; 15-acetoxythapsan-14-al; rearrangement.

Abstract—The benzene extract from the roots of *T. villosa* var. *minor* has afforded the new thapsane derivatives 15-acetoxythapsan-14-al, (1*S*)-1-senecioyloxy-6(14)-thapsen-15-ol, (1*S*,6*R*)-1-senecioyloxy-6,14-epoxythapsan-15-ol and its acetate. Their structures were deduced from spectral data and chemical transformation into known hemiacetalic thapsane derivatives. Acid rearrangement of the epoxythapsane acetate gave a 6-*epi*-formyl derivative and a bicyclo[4.4.0]decadiene derivative.

INTRODUCTION

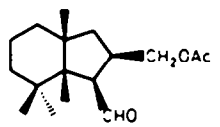
Thapsia villosa var. *minor* is a Mediterranean umbelliferous plant whose roots are rich in sesquiterpenic substances, especially germacrene [1] and thapsane derivatives [2, 3] in contrast with *T. villosa* var. *villosa* in which thapsane derivatives are absent but phenylpropanoids [4, 5] and guaianolides [5, 6] are quite abundant. We would like to report the isolation and characterization of four new non acetalic thapsane derivatives.

The name thapsane [7] was proposed for the skeleton of a new tricyclic hemiacetalic sesquiterpene, like that of **11**, but as we have identified four non acetalic bicyclic thapsane derivatives, besides six other hemiacetalic thapsane esters, we suggested [2] reserving the name thapsane for the bicyclic carbon skeleton **T**.

RESULTS AND DISCUSSION

The neutral benzene extract from the roots of the plant afforded after chromatography on silica gel compounds **1**, **2**, **4** and **5**, besides other germacrene esters and hemiacetalic thapsanes.

The oily compound **1** showed a molecular ion [M]$^+$ at *m/z* 280 in agreement with the empirical formula $C_{17}H_{28}O_3$, and according to its IR spectra an acetate (1750, 1240 cm^{-1}) and an aldehyde (2800, 1720 cm^{-1}) must be present in the molecule. The ^{1}H NMR spectrum confirmed the presence of an acetyl (δ1.92 *s*, 3H) and a formyl group (δ9.68, 1H, *d*, *J* = 4 Hz) and also showed signals of four quaternary methyl groups (δ1.21, *s*, 3H; 1.10, *s*, 3H; 0.95, *s*, 3H; 0.75, *s*, 3H). These data, as well as the number of double bond equivalents, that suggested the presence of two ring systems, and the fact that other thapsane esters were previously isolated from the plant, suggested that **1** also was a thapsane derivative and therefore, the constitution shown was proposed for this substance. The ^{1}H NMR of compound **1** showed a proton signal at δ3.10 (1H, *dd*, *J* = 4.5 and 12 Hz) coupled with the formyl proton and with a vicinal proton at δ2.70 (*m*, 1H). As this multiplet was also coupled with the protons of the primary acetoxyl group (δ4.05 *dd*, *J* = 6 and 10 Hz, 1H; 3.90, *dd*, *J* = 5.5 and 10 Hz, 1H), the aldehyde and the acetoxyl groups were placed on the thapsane carbon atoms C-14 and C-15, respectively. The *cis*-relationship between the formyl and the acetoxymethyl groups was evidenced by the chemical behaviour of **1**. After hydrolysis the expected hydroxyaldehyde could not be isolated but the hemiacetal **6** and/or the dimer **8** were obtained. This acetal **8** is formed from **6** spontaneously or by acid



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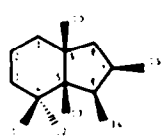
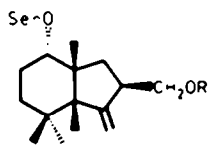


T



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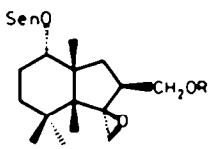
3 R = Ac



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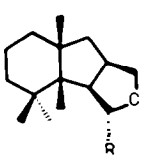
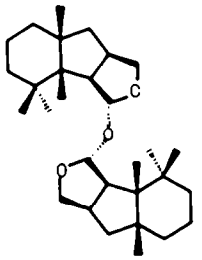
5 R = Ac



6 R: OH
7 R =O



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treatment. Finally, the proposed structure for **1** was confirmed by chemical correlation with the hemiacetalic thapsanes previously isolated. The oxidation of **6** or **8** with Jones reagent gave the γ-lactone **7**, a substance identical in all aspects to that prepared from other natural 14,15-epoxythapsan-14-ol esters [3].

The remaining three natural derivatives **2**, **4** and **5** have a senecioyloxy substituent as evidenced by ^{1}H NMR spectroscopy (δ5.60, *br s*, 1H; 2.15, *br s*, 3H; 1.89, *br s*, 3H) and by mass spectrometry (*m/z*: [M − 100]$^+$, 83 (100), 55).

The crystalline thapsane **2**, with a molecular formula $C_{20}H_{32}O_3$ (M$^+$ at *m/z* 320), showed in the ^{1}H NMR spectrum signals of four quaternary methyl groups (δ1.00, *s*, 6H; 0.86, *s*, 3H; 0.81, *s*, 3H), a terminal methylene group (δ5.00, *d*, *J* = 2 Hz, 1H; 4.82, *d*, *J* = 2 Hz, 1H) and, hydroxymethyl group (δ3.80, *dd*, *J* = 9 and 6 Hz, 1H; 3.47, *t*, *J* = 9 Hz, 1H). In agreement with these data one can assume also a thapsane skeleton for compound **2**. In this substance the hydroxyl group should be placed on C-15 because of the high multiplicity of the H_x proton coupled with the protons of the CH_2OH group, and consequently, the methylene group should be attached to C-6.

The ^{1}H NMR spectrum of **4** showed some of the same signals as seen in the spectrum of **2** but some of them at different fields. The main difference was the absence of the terminal methylene group signal. However, two AB doublets characteristic of a terminal epoxide could be recognized (δ2.85, *d*, *J* = 4 Hz, 1H; 2.72, *d*, *J* = 4 Hz, 1H). This suggested that the compound **4** is the 6,14-epoxide derivative of **2** as it was confirmed by epoxidation with *m*-chloroperbenzoic acid.

Compound **5**, $C_{12}H_{34}O_5$ (M$^+$ at *m/z* 378) showed also IR and ^{1}H NMR spectra similar to those of **4**. The epoxide group was also present in compound **5** however the hydroxyl group was acetylated (ν1740, 1250; δ2.02, *s*, 3H).

The structural relationship of compounds **2**, **4** and **5** was confirmed by chemical transformations. Epoxidation of **2** with *m*-chloroperbenzoic acid yielded exclusively **4** and this substance on acetylation gave **5**. The stereochemistry in the epoxidation of **2** suggested the β-configuration of the epoxide because of the guiding effect of the hydroxyl group [8]. The epoxidation of the acetate **3** was more difficult and the epoxide **5** was obtained together with the product of epoxidation of the senecioyl group.

As mentioned above, compounds **2**, **4** and **5** have a secondary senecioyloxy group. The signal of the geminal proton to the ester group *ca* δ4.75 (1H, apparent triplet, J_{AX+BX} = 14 Hz) showed in these substances a characteristic shape for an equatorial acyloxy group flanked by one $-CH_2-$ and one $-CR_2-$ group in a cyclohexane ring. This suggested that the secondary senecioyloxy substituent should be placed either on C-1 or on C-3. The ester group was finally placed on C-1 on the basis of the chemical correlation with the natural hemiacetalic thapsane **11** whose structure was previously established [3]. In a first experiment to transform the bicyclic derivatives into an hemiacetalic thapsane, compound **2** was acetylated and

Table 1. ^{1}H NMR spectral data for thapsane derivatives*

H	1	2	3	4	5	6	8	9	12	13
1		4.75 *br t* (7)	4.75 *br t* (7)	4.73 *br t* (7)	4.75 *br t* (7)			4.65 *br t* (7)	4.75 *br s*	3.50 *br s*
6	3.10 *dd* (12, 4.5)					2.90 *m*	2.75 *m*	2.70 *m*		
7	2.70 *m*					2.90 *m*	2.75 *m*	2.70 *m*		
10, 11,†	1.21	1.00	1.00	1.15	1.18	1.00	1.00	1.30	1.10	1.05
12, 13	1.10	1.00	1.00	1.02	1.05	0.95	0.95	1.10	1.00	1.00
	0.95	0.86	0.86	0.85	0.86	0.90	0.90	1.05	1.00	0.99
	0.75	0.82	0.84	0.72	0.74	0.85	0.90	0.90	0.86	0.85
14	9.68 *d* (4.5)	5.00 *d* (2)	5.05 *d* (2)	2.85 *d* (4)	2.88 *d* (4)	5.35 *s*	5.25 *s*	9.90 *s*	5.30 *s*	4.70 *s*
		4.82 *d* (2)	4.85 *d* (2)	2.72 *d* (4)	2.70 *d* (4)					
15	4.05 *dd* (10, 6)	3.80 *dd* (10, 6)	4.20 *dd* (10, 6)	3.43 *m*	3.90 *m*	4.15 *br t* (9)	3.90 *br t* (9)	3.80 *m*	4.15 *br t* (9)	3.80 *m*
	3.90 *dd* (10, 5.5)	3.47 *dd* (10, 8)	3.80 *dd* (10, 8)			3.60 *br d* (9)	3.60 *br d* (9)		3.45 *dd* (9, 5)	
Sen		5.60 *m*	5.60 *m*	5.57 *m*	5.60 *m*			5.55 *m*	5.65 *m*	
		2.15 *br s*	2.12 *br s*	2.12 *br s*	2.15 *br s*			2.10 *br s*	2.20 *br s*	
		1.89 *br s*	1.89 *br s*	1.85 *br s*	1.90 *br s*			1.89 *br s*	1.95 *br s*	
Ac	1.92 *s*		2.00 *s*		2.02 *s*			1.91 *s*		
MeO										3.20 *s*

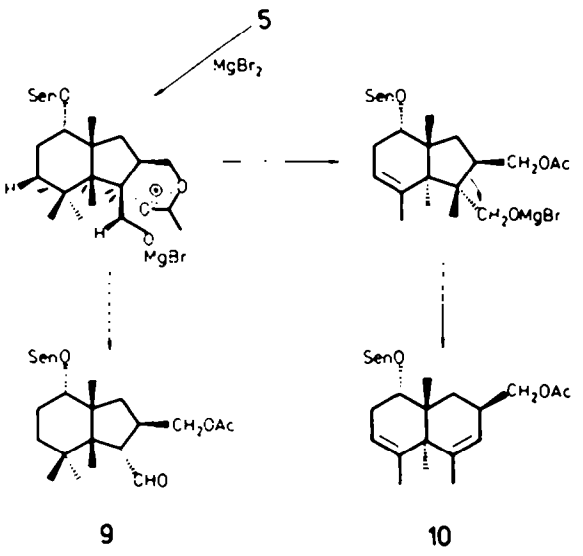
*Recorded at 60 MHz in $CDCl_3$ (**2**, **4**, **5**, **8**) or in CCl_4. δ scale in ppm relative to TMS and *J* in Hz (in parentheses).
†Unassigned signals for the methyl groups 10, 11, 12 and 13.

the hydroboration of the terminal double bond of **3** was attempted without success because of the hindrance of the olefine. Only products resulting from the reduction of the ester groups or from the hydroboration of the senecioyl double bond were obtained. This result led us to try the rearrangement of the terminal epoxide of **5** as an alternative synthesis. The best results were achieved using $MgBr_2$ as Lewis acid. In this case the rearrangement products were chiefly the aldehyde **9** (39%) and another minor by-product (10%) for which the structure **10** was proposed in agreement with the spectral data.

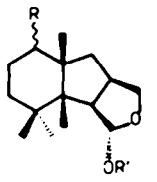
The rearrangement of **3** and the stereochemistry of the products can be explained by opening of the oxirane ring with anchimeric assistance by the acetoxyl group, followed either by a 1,2-H shift to give **9** or by the 1,2-shifts of Me-13 and Me-12 to C-6 and C-5, respectively, followed by deprotonation and ring expansion to give **10** (Scheme 1).

The acetoxymethyl and formyl groups in **2** must be *trans* because after hydrolysis no spontaneous cyclization was observed. After remaining for 12 hr in 0.1 N NaOH–MeOH solution, nearly all the aldehyde **9** was epimerized and transformed into the hemiacetal **12**. To our surprise, compound **12** did not show an equatorial senecioyloxy substituent but an axial one, as deduced from its ^{1}H NMR spectrum: the signal for H-1 appeared as a narrow multiplet (δ4.80, *br s*, $W_{1/2}$ = 7 Hz) characteristic of an equatorial proton. This implies that the formation of the hemiacetal structure takes place with a change in the ring A conformation, namely from $^1C_4$ to $^4C_1$ (steroid like).

The spectral data of compound **12** and those of the natural hemiacetal **11** were similar but these substances differ at least in the configuration of C-1. However, both **11** and **12** were transformed into the same ketone **15**. The hemiacetal **12** was fully hydrolysed and transformed into the methyl ketal **13** to avoid the tendency towards dimerization. The oxidation of **13** with PDC yielded the ketone **15**. The same reaction sequence allowed the natural hemiacetal **11** to be transformed into hydroxyketal **14** and this into the same ketone **15**.



Scheme 1.



	R	R'
11	β-OSen	H
12	α-OSen	H
13	α-OH	Me
14	β-OH	Me
15	=O	Me

The absolute stereochemistry for C-1 and C-6 (1*S*,6*R*), as well as the full structure for the non-hemiacetalic thapsane esters **2**, **4** and **5** were deduced from the above chemical transformations.

EXPERIMENTAL

Extraction and isolation. Plant material was collected, extracted and fractionated as previously reported [1]. Chromatography on silica gel (1 kg) of the neutral part (23 g), eluting with hexane–Et_2O mixtures of increasing polarity, afforded fractions containing the crude thapsanes in the following order: **1**, **5**, **2**, **4**.

15-*Acetoxythapsan*-14-*al* (**1**). Fractions containing **1** (4 g) were further chromatographed on silica gel H-60 (100 g) with hexane–Et_2O (85:15) and afterwards with hexane–EtOAc (95:5) to yield pure **1** (70 mg). Oily; $[\alpha]_D^{20}$ +23.3° ($CHCl_3$; *c* 0.8); IR ν_{max}^{film} cm^{-1}: 2800, 1750, 1720, 1470, 1340, 1240, 1100, 940; EIMS (probe), *m/z* (rel. int.): 280 [M]$^+$ (1), 237 (1), 220 [M − AcOH]$^+$ (5), 191 (6), 178 (25), 135 (25), 108 (100), 107 (99), 95 (80), 81 (60), 79 (80), 55 (70), 43 (90).

(1*S*)-1-*Senecioyloxy*-6(14)-*thapsene*-15-*ol* (**2**). Fractions containing this product (716 mg) were further purified by chromatography on silica gel H-60 (30 g) with hexane–EtOAc (15:5) to afford 120 mg of pure **2**. Mp 133–135° (hexane); $[\alpha]_D$ +11.1° ($CHCl_3$; *c* 2.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 2950, 1700, 1660, 1460, 1390, 1230, 1160, 1090, 1040, 1000, 900, 860; EIMS (probe) *m/z* (rel. int.): 320 [M]$^+$ (2), 302 [M − H_2O]$^+$ (5), 220 [M − SenOH]$^+$ (10), 205 [M − SenOH − H_2O]$^+$ (5), 202 [M − SenOH − H_2O]$^+$ (5), 191 (25), 151 (60), 133 (60), 121 (80), 105 (70), 83 (100), 55 (50).

(1*S*)-15-*Acetoxy*-1-*senecioyloxy*-6(14)-*thapsene* (**3**). Thapsanol **2** (167 mg) was acetylated overnight at room temp. with Ac_2O–pyridine. The product was worked up as usual and crystallized to yield 192 mg of pure **3**: mp 74–76° (hexane); $[\alpha]_D^{20}$ +11.6° ($CHCl_3$; *c* 5.7); IR ν_{max}^{film} cm^{-1}: 2950, 1740, 1710, 1640, 1450, 1380, 1250, 1220, 1140, 1080, 1030, 990, 900; EIMS (probe) *m/z* (rel. int.): 302 [M − AcOH]$^+$ (2), 262 [M − SenOH]$^+$ (3), 202 [M − SenOH − AcOH]$^+$ (35), 133 (95), 83 (100), 57 (80), 43 (50).

(1*S*,6*R*)-1-*Senecioyloxy*-6,14-*epoxythapsan*-15-*ol* (**4**). The fractions containing this substance (3.85 g) were chromatographed on silica gel (200 g) with $CHCl_3$–EtOAc (95:5) to yield 570 mg of **4**: mp 92–94° (hexane); $[\alpha]_D$ −4.11° ($CHCl_3$; *c* 1.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 2950, 1710, 1660, 1460, 1400, 1240, 1160, 1100, 1000, 950, 860; EIMS (probe) *m/z* (rel. int.): 336 [M]$^+$ (2), 236 [M − SenOH]$^+$ (10), 217 (10), 205 (15), 155 (30), 137 (35), 119 (20), 107 (35), 93 (40), 83 (100), 55 (65), 43 (30).

(1*S*,6*R*)-15-*Acetoxyl*-1-*senecioyloxy*-6,14-*epoxythapsane* (**5**).

The crude material (600 mg) was chromatographed on silica gel (40 g) with hexane $CHCl_3$–EtOAc (70:25:5) to yield 143 mg of crystalline compound **5**: mp 141–143° (hexane); $[\alpha]_D^{20}$ +10.0° ($CHCl_3$; *c* 0.3); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1740, 1710, 1660, 1480, 1400, 1380, 1250, 1160, 1110, 1050, 1000; EIMS (probe) *m/z* (rel. int.): 378 $[M]^+$ (2), 318 (3), 278 (3), 218 (8), 203 (8), 133 (35), 83 (100).

14,15-*Epoxythapsan*-14-*ol* (**6**) *and di*(14,15-*epoxythapsan*-14-*yl*)*ether* (**8**). Thapsanal (**1**, 80 mg) was left in 0.1 N NaOH MeOH for 1 hr at room temp. Usual work up yielded a mixture (60 mg) of products **6** and **8**. Chromatography on silica gel (10 g) with hexane Et_2O (4:1) allowed the separation of **6** (19 mg) from **8** (29 mg). Product **6**: mp 110° (decomp); $[\alpha]_D$ −62.0° ($CHCl_3$; *c* 1.0); IR ν_{max}^{nujol} cm^{-1}: 3400, 2900, 1450, 1380, 1210, 1120, 1040, 980, 920. Product **8**: mp 198–200° (hexane); $[\alpha]_D$ −128.4° ($CHCl_3$; *c* 1.5); IR ν_{max}^{nujol} cm^{-1}: 2900, 1450, 1380, 1130, 1100, 1050, 970, 940, 930, 920. EIMS (probe) *m/z* (rel. int.): 237 (8), 221 (30), 139 (15), 106 (20), 83 (40), 69 (100), 55 (20).

On heating the hemiacetal **6** it spontaneously evolves to the dimeric acetal **8**. Oxidation of a mixture of **6** and **8** with Jones reagent in the usual way gave thapsanolide **7** previously described [3].

Rearrangement of **5** *with* $MgBr_2$ *to yield* 15-*acetoxyl*-1-*senecioyloxy*-6-*epithapsan*-14-*al* (**9**). Epoxide **5** (1.92 g) in Et_2O (60 ml) and C_6H_6 (30 ml) with $MgBr_2$ (obtained from 300 mg Mg and 2.1 g $HgBr_2$) was refluxed for 3 days. Work up yielded a mixture (1.90 g) which was chromatographed (60 g silica gel; hexane–Et_2O, 8:2) yielding 757 mg of the pure compound **9**: mp 116–118° (hexane); $[\alpha]_D$ −30.0° ($CHCl_3$; *c* 0.4); IR ν_{max}^{film} cm^{-1}: 2950, 2700, 1740, 1710, 1650, 1450, 1380, 1230, 1150, 1080, 1040, 1000, 850; EIMS (probe) *m/z* (rel. int.): 378 $[M]^+$ (1), 278 [M − AcOH]$^+$ (1), 218 [M − AcOH − SenOH]$^+$ (1), 149 (10), 121 (10), 107 (15), 83 (100), 69 (15), 55 (20).

4-*Acetoxymethyl*-7-*senecioyloxy*-1,2,6,10-*tetramethylbicyclo*-[4.4.0] *deca*-2,9-*diene* (**10**). This substance (65 mg) was present in the less polar fraction of the preceding chromatography. After chromatography on silica gel (10 g, hexane–Et_2O, 95:5) pure **10** was isolated. Oily; $[\alpha]_D$ +24.6; IR ν_{max}^{film} cm^{-1}: 2950, 1745, 1720, 1650, 1450, 1380, 1350, 1230, 1150, 1080, 1050, 1020, 900, 850; ^1H NMR (60 MHz, CCl_4): δ 5.60 (*s*, 1H), 4.90 (*m*, 3H), 4.20 (*dd*, *J* = 6 and 11 Hz, 1H), 3.95 (*dd*, *J* = 7 and 11 Hz, 1H), 2.17 (*s*, 3H), 2.00 (*s*, 3H), 1.90 (*s*, 3H), 1.65 (*s*, 3H), 1.60 (*s*, 3H), 1.11 (*s*, 3H), 0.89 (*s*, 3H); EIMS (probe) *m/z* (rel. int.): 360 $[M]^+$ (1), 300 [M − AcOH]$^+$ (2), 260 [M − SenOH − AcOH]$^+$ (10), 200 [M − AcOH − SenOH]$^+$ (50), 185 (80), 83 (100), 55 (30), 43 (20).

1-*Senecioyloxy*-14,15-*epoxythapsan*-14-*ol* (**12**). The acetoxyaldehyde **9** (110 mg) was kept in 0.1 N NaOH–MeOH (2 ml) for 12 hr at room temp. Work up and chromatography (silica gel, hexane–Et_2O, 6:4) afforded 40 mg of the pure thapsanol **12**: Oily; $[\alpha]_D^{20}$ −47.6° ($CHCl_3$; *c* 2.1); IR ν_{max}^{film} cm^{-1}: 3400, 2950, 1700, 1650, 1450, 1380, 1220, 1150, 1080, 1000, 920, 850, 750; EIMS (probe) *m/z* (rel. int.): 318 (2), 199 (15), 175 (10), 137 (35), 123 (75), 107 (30), 83 (100), 55 (40), 43 (10).

14-*Methoxy*-14,15-*epoxythapsan*-1-*ol* (**13**). To a soln of **9** (750 mg) in MeOH (50 ml), 0.06 N NaOH–MeOH (1.5 ml) was added. After refluxing for 3 hr the reaction mixture was concd to 5 ml and more 2 N NaOH–MeOH (2 ml) was added. Refluxing was continued overnight. Work up yielded 527 mg of a crude product which was dissolved in MeOH (40 ml) with a trace of *p*-TsOH. Work up and chromatography on silica gel (hexane–Et_2O, 1:1) yielded 335 mg of crystalline compound **13**: mp 130–132° (Et_2O); $[\alpha]_D^{20}$ −77.8° ($CHCl_3$; *c* 5.6); IR ν_{max}^{film} cm^{-1}: 3400, 2900, 1450, 1380, 1100, 1070, 1030, 1000, 920; EIMS (probe) *m/z* (rel. int.): 237 (10), 219 (4), 190 (4), 175 (15), 154 (15), 149 (15), 121 (30), 108 (100), 93 (75), 55 (25).

Product **13** (100 mg) was oxidized overnight in 5 ml CH_2Cl_2 with PDC (100 mg). Work up yielded 80 mg of a product whose physical properties were identical with those previously reported for **15** [3].

REFERENCES

1. Pascual Teresa, J., Morán, J. R., Hernández, J. M. and Grande, M. (1985) *Phytochemistry* **24**, 1779.
2. Pascual Teresa, J., Morán, J. and Grande, M. (1985) *Chem. Letters* 865.
3. Pascual Teresa, J., Morán, J. R., Fernández, A. and Grande, M. (1986) *Phytochemistry* **25**, 703.
4. Pascual Teresa, J., Pascual, M., Arias, A., Hernández, J. M., Morán, J. R. and Grande, M. (1985) *Phytochemistry* **24**, 1773.
5. Pascual Teresa, J., Morán, J. R., Hernández, J. M. and Grande, M. (1985) *Phytochemistry* **24**, 2071.
6. Christensen, S. B., Norup, E., Rasmussen, U. and Madsen, J. D. (1984) *Phytochemistry* **23**, 1659.
7. Lemmich, E., Jensen, B. and Rasmussen, U. (1984) *Phytochemistry* **23**, 809.
8. Henbest, H. B. and Wilson, R. A. L. (1957) *J. Chem. Soc.* 1958.