Phytochemistry, Vol. 26, No. 2, pp. 445–448, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

HUMULENE DERIVATIVES FROM *HETEROTHECA VILLOSA*

J. JAKUPOVIC, R. BOEKER, F. BOHLMANN, R. M. KING* and H. ROBINSON*

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, F.R.G.; *Smithsonian Institution, Department of Botany, Washington, DC 20560, U.S.A.

(*Received* 19 *May* 1986)

Key Word Index—*Heterotheca villosa*; Compositae; sesquiterpenes; humulene derivatives; umbelliferone derivative.

Abstract—The aerial parts of *Heterotheca villosa* afforded, in addition to known compounds, eleven new humulene derivatives. The roots gave a new umbelliferone derivative. The structures were elucidated by high-field NMR spectroscopy.

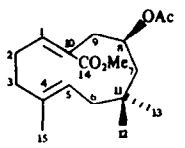
INTRODUCTION

So far, the small North American genus *Heterotheca* (Compositae, tribe Astereae) has given mainly cadinene derivatives [1–5] and in one case the essential oil was analysed [6]. We have now studied the constituents of *H. villosa* (Pursh.) Shinners. The aerial parts gave caryophyllenepoxide, spathulenol, carissone [7], 5β,6α-epoxycaryophyllen-13-oic acid [8] and eleven new humulene derivatives, the lactone **4** and ten acids which were transformed to the methyl esters **1–3** and **5–11**. The roots afforded 3-isovaleryl-4-hydroxyacetophenone and the umbelliferone derivative **12**. The structure elucidation is discussed in this paper.

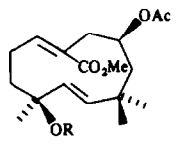
RESULTS AND DISCUSSION

We start with the discussion of the structure of **4**. The IR spectrum showed the presence of a hydroxyl group. Together with the ^{1}H NMR spectrum (Table 1), two bands at 1730 and 1710 cm^{-1} indicated an acetate and an unsaturated ester group. Acetylation gave the diacetate **4a**. In the ^{1}H NMR spectra of **4** and **4a**, all the signals could be assigned by spin decoupling. Starting with the low-field signal at δ6.32 in the spectrum of **4**, which obviously was due to H-1, the sequences of most of the carbons could be determined. The remaining quaternary carbons (C-4 and C-11) could be placed only between C-3 and C-5 and between C-6 and C-7. Accordingly, the presence of a humulene derivative was very likely as the remaining methyl groups and the oxygen function were needed to form the quaternary carbons. This assumption and the stereochemistry were established by NOE difference spectroscopy and considerations of models. Clear effects were observed between H-13, H-6α and H-8, between H-12 and H-7α, between H-15 and H-3α, between H-5, H-7β, H-3β and H-1, between H-1 and H-9β, and between H-8, H-6α and H-13. This, and *W*-couplings between H-13 and H-7β, between H-7α and H-6β, and between H-12 and H-6α, required a conformation with the lactone ring below the plane and the oxygen functions situated equatorially. The ^{13}C NMR spectrum (see Experimental) also supported the structure. The relative positions of the oxygen functions followed from the chemical shifts in the spectrum of **4**, especially as the protons at C-5 and C-8 could easily be differentiated by their couplings. In the mass spectrum of **4**, no molecular ion could be detected. However, by chemical ionization, a clear $[M+1]^+$ peak was observed counting for $C_{17}H_{26}O_5$.

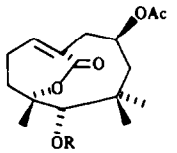
The ^{1}H NMR spectrum of **1** (Table 1), molecular formula $C_{18}H_{28}O_4$, indicated the presence of a 1(10),4-diene, again with an acetoxyl group at C-8. Inspection of models showed that the observed couplings required the proposed stereochemistry of the double bonds. Furthermore, the chemical shift of H-1 indicated a 1,10*Z*-configuration of the double bond.



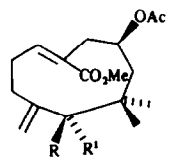
1
2 (4β, 5α-epoxide)



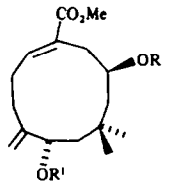
3 R = OH
3a R = H



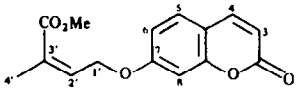
4 R = H
4a R = Ac



	5	6	7	8
R	OH	H	H	H
R¹	H	OH	OOH	OAc



	9	10	11
R	iVal	Tigl	Tigl
R¹	H	H	OH



12

Table 1. ^{1}H NMR spectral data of compounds **1**–**6**, **3a**, **4a** and **8**–**11** (400 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**	**3**	**3a**	**4**	**4a**	**5**	**6**	**8**	**9**	**10**	**11**
H-1	6.00 *br dd*	6.17 *br dd*	6.03 *br dd*	5.82 *br dd*	6.32 *ddd*	6.46 *ddd*	6.36 *br dd*	6.08 *ddd*	6.13 *br dd*	6.95 *dd*	6.95 *dd*	6.93 *br dd*
H-5	5.04 *br dd*	2.84 *d*	5.26 *d*	5.30 *d*	4.49 *d*	6.01 *d*	4.14 *dd*	4.04 *dd*	5.08 *br dd*	4.13 *dd*	4.13 *dd*	4.33 *dd*
H-6α	1.81 *dd*	1.30 *dd*	5.10 *d*	5.24 *d*	1.77 *br d*	1.75 *br d*	1.52 *br dd*	1.79 *dd*	1.74 *br dd*	2.00 *br dd*	2.02 *br dd*	1.84 *br dd*
H-6β	1.91 *br dd*	1.53 *br d*			1.04 *ddd*	1.35 *ddd*	1.42 *dd*	1.16 *ddd*	1.44 *m*	1.36 *br dd*	1.37 *br dd*	1.49 *br dd*
H-7α	1.63 *br d*	1.50 *br dd*	1.61 *m*	1.69 *m*	1.52 *br d*	1.50 *br ddd*	1.47 *br dd*	1.41 *ddd*	1.44 *m*	1.56 *ddd*	1.60 *ddd*	1.60 *m*
H-7β	1.35 *br dd*	1.72 *br d*			1.82 *dd*	1.90 *br dd*	1.97 *br d*	1.80 *br d*	1.83 *br d*	1.71 *br d*	1.73 *br d*	
H-8	4.86 *ddd*	4.90 *br ddd*	4.64 *dddd*	4.55 *dddd*	4.90 *dddd*	4.89 *dddd*	5.10 *br ddd*	4.84 *ddd*	4.82 *br ddd*	5.04 *br ddd*	5.08 *br ddd*	5.08 *m*
H-9α	2.36 *br dd*	2.41 *br dd*	2.51 *br d*	2.61 *br d*	3.01 *dddd*	3.04 *dddd*	2.55 *br dd*	2.51 *br dd*	2.53 *br d*	2.73 *br dd*	2.75 *br dd*	2.70 *m*
H-9β	2.31 *dd*	2.48 *dd*	2.32 *dd*	2.26 *dd*	2.16 *dd*	2.20 *dd*	2.42 *dd*	2.34 *dd*	2.39 *dd*	2.52 *dd*	2.61 *dd*	
H-12	0.93 *s*	0.99 *s*	0.96 *s*	0.98 *s*	0.97 *s*	0.74 *s*	0.95 *s*	1.01 *s*	0.86 *s*	1.01 *s*	1.01 *s*	0.97 *s*
H-13	0.86 *s*	0.93 *s*	0.94 *s*	0.94 *s*	0.92 *s*	0.91 *s*	0.91 *s*	0.79 *s*	0.81 *s*	0.84 *s*	0.82 *s*	0.84 *s*
H-15	1.40 *br s*	1.15 *s*	1.36 *s*	1.24 *s*	1.34 *s*	1.33 *s*	5.05 *br s* 4.90 *br s*	5.10 *br s* 4.91 *br s*	5.00 *br s* 4.91 *br s*	5.14 *br s* 4.98 *br s*	5.14 *br s* 4.99 *br s*	5.22 *br s* 2.20 *br s*
OMe	3.73 *s*	3.77 *s*	3.76 *s*	3.80 *s*	—	—	3.74 *s*	3.75 *s*	3.80 *s*	3.73 *s*	3.71 *s*	3.74 *s*
OR	2.01 *s*	2.03 *s*	2.03 *s*	2.02 *s*	1.99 *s*	2.03 *s*	2.03 *s*	2.01 *s*	2.02 *s*	2.14 *m*	6.83 *qq*	6.84 *qq*
			7.39 *s* (OOH)			2.00 *s*			1.98 *s*	2.08 *m*	1.82 *br s*	1.82 *br s*
										0.96 *d*	1.78 *br d*	1.79 *br d*
										0.95 *d*		

J (Hz): compound **1**: 1,2α = 10.5; 1,2β = 7; 5,6α = 5.5; 5,6β = 8.5; 6α,6β = 7α,7β = 16; 7β,8 = 8,9β = 9; 8,9α = 4; 9α,9β = 13.5; compound **2**: 1,2α = 12; 1,2β = 4; 5,6α = 10; 5,6β ~ 0.5; 6α,6β = 15; 7α,7β = 16; 7α,8 = 9; 8,9α = 5; 8,9β = 10; 9α,9β = 14; compounds **3** and **3a**: 1,2α = 9; 1,2β = 8; 5,6 = 16; 7α,8 = 7β,8 = 8,9α = 3.5; 8,9β = 12; 9α,9β = 13; compounds **4** and **4a**: 1,2α = 9; 1,2β = 5; 1,9α = 2; 2β,9α = 2.5; 5,6β = 8.5; 6α,6β = 7α,7β = 16; 6β,7α = 1.5; 7α,8 = 2.5; 7β,8 = 8,9α = 4; 8,9α = 9α,9β = 12; compound **5**: 1,2α = 5; 1,2β = 10; 5,6α = 9; 5,6β = 3.5; 6α,6β = 15; 7α,7β = 17; 7α,8 = 5; 8,9α = 4; 8,9β = 11; 9α,9β = 13; compounds **6** and **8**: 1,2α = 6; 1,2β = 10; 1,9α = 1.5; 5,6α = 2.5; 5,6β = 8; 6α,6β = 7α,7β = 16; 6β,7α = 2; 7α,8 = 6.5; 8,9α = 4; 8,9β = 12; 9α,9β = 14; compounds **9**–**11**: 1,2α = 1,2β = 8; 5,6α = 4; 5,6β = 6; 6α,6β = 7α,7β = 9α,9β = 15; 6β,7α = 1; 7α,8 = 8.5; 8,9α = 6; 8,9β = 7.

All the data of **2** showed that this ester was the corresponding 4β,5α-epoxide of **1**. In agreement with considerations of models, the missing coupling $J_{5,6\alpha}$ was due to an almost 90° angle. Most likely, the acid corresponding to **1** is the precursor of **4**.

The ^{1}H NMR spectrum of **3** (Table 1) indicate that this acetate was a hydroperoxide (δ7.39 *s*). Reaction with triphenylphosphine afforded **3a**. The presence of an *E*-configuration for the disubstituted double bond followed from the coupling of the olefinic proton (H-5 and H-6). The remaining signals were close to those of **1** except that of H-15. As in similar cases, the hydroperoxide caused small downfield shifts of neighbouring protons.

Compound **7** was an isomer of **3** where the Δ^5-double bond was replaced by a $\Delta^{4(15)}$-double bond. Triphenylphosphine reduction afforded the carbinol **6**, which was also isolated from the extract. The ^{1}H NMR spectrum of the latter (Table 1) and that of the isomer **5** were in part similar but showed some small differences in the chemical shifts and also the couplings were slightly different. In particular, the couplings of H-5 indicated that **5** and **6** were most likely epimeric at C-5. All signals could be assigned by spin decoupling and NOE difference spectroscopy established the stereochemistry. Thus in the case of **5** clear effects were observed between H-8, H-5 and H-1 as well as between H-13, H-5 and H-8. In the case of **6**, clear effects were observed between H-13, H-8, H-6β and H-7α, between H-12, H-5, H-13 and H-6β, between H-5 and H-7β, between H-8 and H-6α, and between H-1 and H-8. Inspection of models showed that these results required the proposed configurations at C-5, and that both esters had a preferred conformation with C-14 above and C-15 below the plane. This was supported by *W*-couplings in the case of **6** between H-6β and H-7α, between H-13 and H-7β, and between H-12 and H-6α. The ^{1}H NMR data of **8** (Table 1) clearly showed that this ester was the acetate of **6**, which could be established by acetylation of the latter.

The ^{1}H NMR spectra of **9** and **10** (Table 1) differed only in the signals of the ester groups, and compound **11** again was a hydroperoxide and could be reduced to **10**. In the spectra of **9** and **10** the H-1 signal was at δ6.95. Accordingly, the 1(10)-double bond had the *E*-configuration. The remaining signals were close to those of **6** although all showed small shift differences and also several couplings were altered. The stereochemistry was established by NOE difference spectroscopy. Thus, in the case of **9**, clear effects were observed between H-13, H-8, H-12, H-6α and H-6β, between H-12, H-5, H-13 and H-7β, between H-5, H-13 and H-7β, and between H-8 and H-6α. In the preferred conformation both C-14 and C-15 were below the plane.

The structure of **12** was deduced from the ^{1}H NMR spectrum (see Experimental). In addition to the typical signals of umbelliferone ether, the signals of an angelate with an oxygen function at C-4 were present. From the chemical shift of H-2′ the *Z*-configuration of the double bond was deduced.

Prenylated umbelliferone derivatives are also present in *Haplopappus* species [9–11], which are also placed in the subtribe Solidagininae. So far, humulene derivatives have not been reported from genera of this subtribe. These compounds seem to be relatively rare in the Compositae. However, further investigations may show whether these compounds are more widespread.

EXPERIMENTAL

The air-dried plant material (voucher RMK 9492, deposited at the U.S. National Herbarium, Washington, collected in Colorado in July 1985) was extracted as reported previously [12]. The extract (7 g) of the aerial parts (800 g) gave by CC (silica gel) 10 mg caryophyllenepoxide, 5 mg spathulenol and a polar fraction (Et_2O and Et_2O–MeOH, 9:1) which was separated by HPLC (RP 8, MeOH–H_2O, 3:2, *ca* 100 bar) affording seven fractions. Fraction 1 contained 2 mg **4** (R_t 7.3 min). Fraction 2 (R_t 9.5 min) was a mixture of acids which were transformed into their methyl esters (CH_2N_2 in Et_2O). Prep. TLC (Et_2O–petrol, 3:2) gave 6 mg **6** (R_f 0.26), 1.5 mg **3** (R_f 0.35) and 1 mg **7** (R_f 0.30). Fraction 3 (R_t 10.5 min) was also a mixture of acids. Prep. TLC of the methyl esters (Et_2O–petrol, 3:2) gave 1.5 mg **5** (R_f 0.52), 1.5 mg **2** (R_f 0.59) and 2 mg **1** (R_f 0.81). Fraction 4 (R_t 13.2 min) gave on prep. TLC (Et_2O–petrol, 7:3) 3 mg carissone and 4 mg 5β,6α-epoxycaryophyllen-13-oic acid. Fraction 5 (R_t 21.4 min) gave after addition of CH_2N_2 and prep. TLC (Et_2O–petrol, 7:3) 3 mg **10**. Fraction 6 (R_t 24.2 min) gave after addition of CH_2N_2 6 mg **8** and fraction 7 (R_t 26.2 min) afforded after addition of CH_2N_2 and prep. TLC (Et_2O–petrol, 3:2, two developments) 0.5 mg **9** (R_f 0.23) and 1 mg **11** (R_f 0.34).

The extract (0.5 g) of the roots (100 g) gave on CC a polar fraction (Et_2O) which gave on prep. TLC (Et_2O–petrol, 2:3) 4 mg 3-isovaleryl-4-hydroxyacetophenone and a crude fraction which gave on HPLC (MeOH–H_2O, 3:2) 10 mg **12** (R_t 0.8 min). Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

*Methyl-8β-acetoxyhumula-*1(10)*Z*,4*E-dien-*14-*oate* (**1**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1250 (OAc), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 308.199 [M]$^+$ (3.5) (calc. for $C_{18}H_{28}O_4$: 308.199), 276 [M − MeOH]$^+$ (3.5), 248 [M − HOAc]$^+$ (25), 233 [248 − Me]$^+$ (12), 216 [248 − MeOH]$^+$ (18), 189 [248 − CO_2Me]$^+$ (42), 121 (44), 61 (100).

*Methyl-8β-acetoxy-4β,5α-epoxyhumul-*1(10)*Z-en-*14-*oate* (**2**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1250 (OAc), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 264.173 [M − HOAc]$^+$ (22) (calc. for $C_{16}H_{24}O_3$: 264.173), 249 [264 − Me]$^+$ (17), 232 [264 − MeOH]$^+$ (28), 189 (41), 121 (42), 55 (100).

*Methyl-8β-acetoxy-4β-hydroperoxyhumula-*1(10)*Z-*5*E-dien-*14-*oate* (**3**). Colourless oil; ^{1}H NMR: see Table 1. Addition of triphenylphosphine in $CDCl_3$ afforded after standing for 10 min at20° and prep. TLC (Et_2O–petrol, 1:1) **3a**; MS *m/z* (rel. int.): 264.172 [M − HOAc]$^+$ (10) (calc. for $C_{16}H_{24}O_3$: 264.173), 246 [264 − H_2O]$^+$ (10), 232 [264 − MeOH]$^+$ (14), 189 (20), 57 (100).

*8β-Acetoxy-5α-hydroxyhumul-*1(10)*Z-en-*4α,14-*olide* (**4**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1240 (OAc), 1710 (C=CCO_2R); CIMS *m/z* (rel. int.): 311 [M + 1]$^+$ (55), 251 [311 − HOAc]$^+$ (100); ^{13}C NMR ($CDCl_3$, C-1–C-15): δ137.7 *d*, 23.6 *t*, 39.3 *t*, 85.4 *s*, 67.9 *d*, 42.1 *t*, 45.3 *t*, 71.0 *d*, 37.5 *t*, 135.3 *s*, 32.8 *s*, 23.6 *q*, 28.9 *q*, 169.4 *s*, 30.0 *q*; OAc: 169.5 *s*, 21.5 *q*. Acetylation (Ac_2O, 1 hr, 70°) gave **4a**, colourless oil; ^{1}H NMR: see Table 1.

*Methyl-8β-acetoxy-5β-hydroxyhumula-*1(10)*Z-*4(15)*-dien-*14-*oate* (**5**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510 (OH), 1730, 1240 (OAc), 1710 (C=CCO_2R); MS *m/z* (rel. int.): 264.173 [M − HOAc]$^+$ (26) (calc. for $C_{16}H_{24}O_3$: 264.173), 249 (23), 232 (24), 121 (56), 83 (100).

*Methyl-8β-acetoxy-5α-hydroxyhumula-*1(10)*Z,*4(15)*-dien-*14-*oate* (**6**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1740, 1240 (OAc), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 264.172 [M − HOAc]$^+$ (24) (calc. for $C_{16}H_{24}O_3$: 264.173), 249 (24), 232 (27), 121 (54), 83 (100). Acetylation (Ac_2O, 1 hr, 70°) gave **8**, identical with the natural product.

Methyl-8β-acetoxy-5α-hydroperoxyhumula-1(10)Z,4(15)-dien-14-oate (**7**). Colourless oil; ^{1}H NMR: see Table 1. Addition of triphenylphospine gave **6**, identical with the natural compound.

Methyl-5α,8β-diacetoxyhumula-1(10)Z,4(15)-dien-14-oate (**8**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1750 (OAc), 1720 (C=CCO$_2$R); CIMS *m/z* (rel. int.): 367 [M + 1]$^+$ (26), 307 [367 − HOAc]$^+$ (56), 247 [307 − HOAc]$^+$ (100).

Methyl-8β-isovaleryloxy-5α-hydroxyhumula-1(10)E,4(15)-dien-14-oate (**9**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1720 (CO$_2$R); CIMS *m/z* (rel. int.): 367 [M + 1]$^+$ (26), 265 [367 − RCO$_2$H] (52), 247 [265 − H$_2$O]$^+$ (100).

Methyl-8β-tigloyloxy-5α-hydroxyhumula-1(10)E,4(15)-dien-14-oate (**10**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1720 (CO$_2$R); MS *m/z* (rel. int.): 364 [M]$^+$ (0.2) 264.173 [M − RCO$_2$H]$^+$ (7) (calc. for C$_{16}$H$_{24}$O$_3$: 264.173), 249 (3), 246 (7), 232 (5), 121 (7), 83 (100), 55 [83 − CO]$^+$ (49).

Methyl-8β-tigloyloxy-5α-hydroperoxyhumula-1(10)E,4(15)-dien-14-oate (**11**). Colourless oil; MS *m/z* (rel. int.): 246 [M − H$_2$O$_2$, RCO$_2$H]$^+$ (4), 231 (3), 83 (100), 55 [83 − CO]$^+$ (38). Addition of triphenylphosphine gave **10**, identical with the natural compound.

Umbelliferone-[3-carbomethoxybut-2Z-enyl(1)]-ether (**12**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750, 1620 (coumarin), 1720 (C=CCO$_2$R); MS *m/z* (rel. int.): 274 [M]$^+$ (4.5), 243 [M − OMe]$^+$ (16), 113 [MeO$_2$CC(Me)=CHCH$_2$]$^+$ (100), 81 [113 − MeOH]$^+$ (33); ^{1}H NMR ($CDCl_3$): δ 6.26 (*d*, H-3), 7.63 (*d*, H-4), 7.37 (*d*, H-5), 6.85 (*dd*, H-6), 6.82 (*d*, H-8), 5.07 (*dq*, H-1'), 6.20 (*tq*, H-2'), 1.98 (*dt*, H-4'), 3.80 (s, OMe) [*J* (Hz): 3,4 = 9.5; 5,6 = 8.5; 6,8 = 2; 1',2' = 4.5; 1',4' = 2',4' = 2].

REFERENCES

1. Bohlmann, F., Wolfrum, C., Jakupovic, J., King, R. M. and Robinson, H. (1985) *Phytochemistry* **24**, 1101.
2. Bohlmann, F., Gupta, R. K., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2982.
3. Bohlmann, F., Zdero, C., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 1675.
4. Bohlmann, F. and Zdero, C. (1976) *Chem. Ber.* **109**, 2021.
5. Bohlmann, F. and Zdero, C. (1979) *Phytochemistry* **18**, 1185.
6. Lincoln, D. E. and Lawrence, B. M. (1984) *Phytochemistry* **23**, 933.
7. Ayer, W. A. and Taylor, W. T. (1955) *J. Chem. Soc.* 2027.
8. Jakupovic, J., Ellmauerer, E., Bohlmann, F., King, R. M. and Robinson, H. (1986) *Phytochemistry* **25**, 1927.
9. Bohlmann, F. and Jakupovic, J. (1979) *Phytochemistry* **18**, 1367.
10. Bohlmann, F., Fritz, U., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 1749.
11. Chiang, M. T., Bittner, M., Silva, M., Mondaca, A., Zemelman, R. and Sammes, P. G. (1982) *Phytochemistry* **21**, 2753.
12. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1979.