Phytochemistry, Vol 27, No 11, pp 3619–3624, 1988
Printed in Great Britain.

0031-9422/88 $3.00+0.00
© 1988 Pergamon Press plc

ω-OXYGENATED PRENYLATED COUMARINS FROM *FERULA COMMUNIS*

GIOVANNI APPENDINO, SILVIA TAGLIAPIETRA, PIERLUIGI GARIBOLDI,* GIAN MARIO NANO and VINCENZO PICCI†

Dipartimento di Scienza e Tecnologia del Farmaco, C so Raffaello 31, 10125 Torino, Italy; *Dipartimento di Scienze Chimiche, Via S Agostino 1, 62032 Camerino (Mc), Italy, †Laboratorio di Botanica Farmaceutica, Facoltà di Farmacia, Via Muroni 23/A 07100 Sassari, Italy.

(*Received* 22 *February* 1988)

Key Word Index—*Ferula communis*; Umbelliferae, prenylated coumarins; ferulenol, ferprenin.

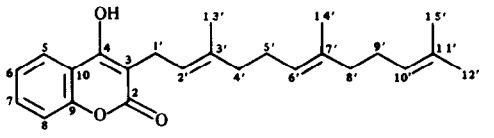
Abstract—From the toxic variety of *Ferula communis*, derivatives of the prenylated coumarins ferulenol and ferprenin bearing an oxygen function (hydroxyl, acetoxyl, aldehydic carbonyl) at the ω-position have been isolated. The structures of the coumarins were established by spectral methods and by chemical reactions. Photooxygenation of ferulenol and (*E*) ω-hydroxyferulenol gave *o*-hydroxyphenylglyoxylic esters, resulting from the oxidative decarbonylation of the 4-hydroxycoumarinic nucleus and loss of the prenyl side chain Ethyl *o*-hydroxyphenylglyoxylate was also isolated from the plant extract, suggesting that a reaction of this type might be responsible for the degradation of the prenylated coumarins in plant samples and extracts of *Ferula communis*.

INTRODUCTION

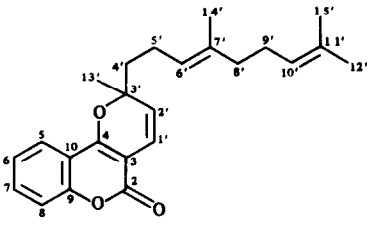
It has been known for a long time that the consumption of *Ferula communis* L. can cause in livestock an often lethal disease known as ferulosys [1–3]. Cases of human poisoning from ingestion of *F. communis* have also been reported [4, 5]. Ferulosys shows haemorrhagic symptoms similar to those of poisoning from fermented sweet clover, but extracts of the plant have also been shown to elicit toxic effects on the central nervous system and the liver [2, 3].

In spite of the wide geographical distribution of *F communis*, ferulosys appears to be limited to a few areas of the western Mediterranean region, especially Sardinia, where this poisoning was first described and systematically studied [1–3]. It was early recognized that two populations of *F. communis* exist in Sardinia, since only the plants from certain parts of the island were toxic to livestock [2, 3]. A wide range of toxicity has also been reported to be present within the toxic population [2, 3].

A recent investigation of *F. communis* from Sardinia has shown the presence of two distinct chemical races, one containing sesquiterpene esters, and the other prenylated coumarins [6]. Biological tests established that only the latter is toxic [7] The prenylated coumarins ferulenol (**1**) [6] and ferprenin (**2**) [8] were isolated from the toxic variety, and both showed haemorrhagic activity *in vivo* [7]. Besides these compounds, more polar coumarin derivatives were also present, in rather variable amounts, in the extracts. Our interest in these products was prompted by the observation that samples of *F. communis* lacking **1** and **2** and containing exclusively the more polar coumarins, were still highly toxic to experiment animals (M. Aragno and G. Ugazio, personal communication) We present here the structure elucidation of these products.



1

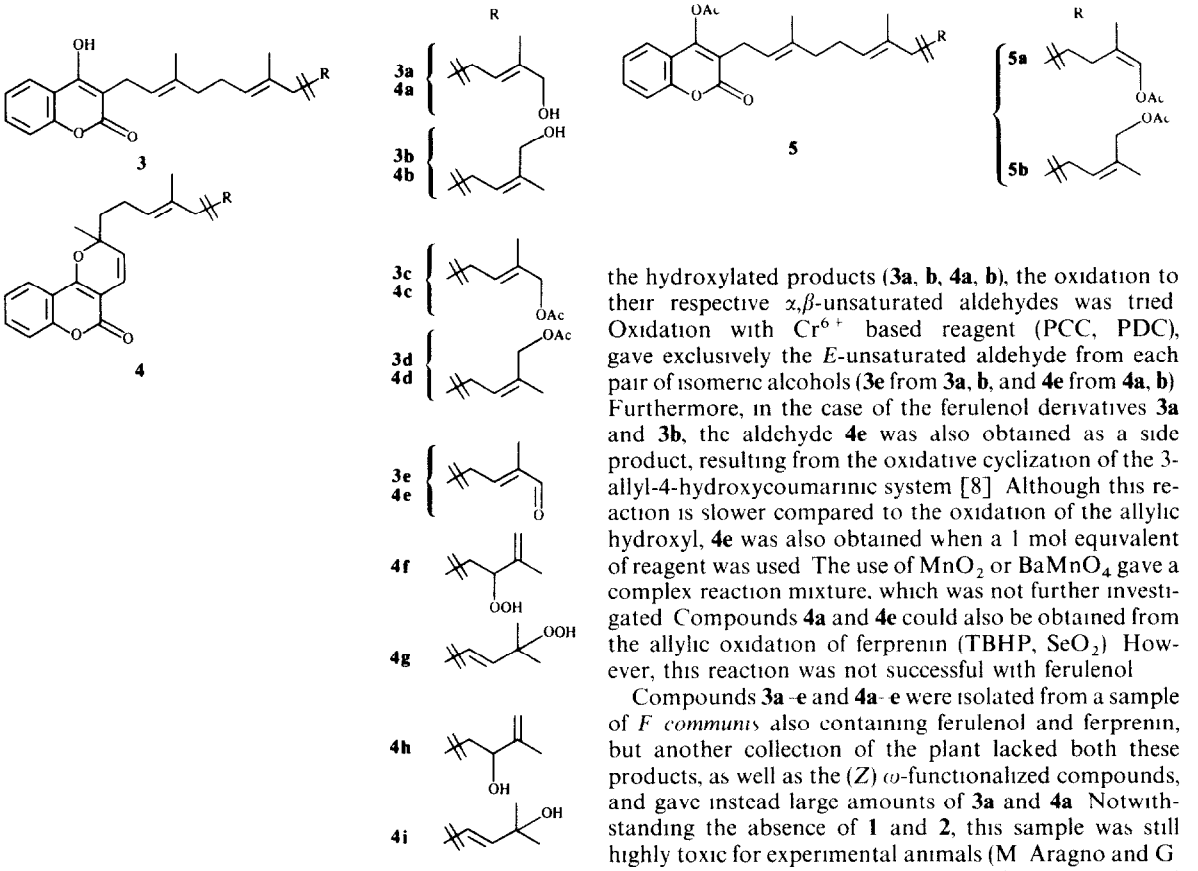


2

RESULTS AND DISCUSSION

The compounds isolated (**3a–e** and **4a–e**) are derivatives of ferulenol (**1**) and ferprenin (**2**) bearing an oxygen function (hydroxyl, acetoxyl, aldehydic carbonyl) at the ω-position. In the case of the hydroxy- and acetoxyderivatives both pairs of geometrical isomers were isolated; for the aldehydic compounds, exclusively the *E*-isomers were found. All compounds are oils, and were obtained in pure form using a combination of column chromatography and preparative HPLC.

As a result of having both isomers at our disposal, the structural assignment of the hydroxy- (**3a, b**; **4a, b**) and acetoxyderivatives (**3c, d**; **4c, d**) by spectral means was straightforward We had already isolated **3a** from a Sardinian collection of *F. communis* [6], but its structural assignment was ambiguous, since it was partly based on the oxidation to the corresponding aldehyde, a reaction which is not stereospecific (see below); compound **3b** has recently been reported from a collection of *F. communis*



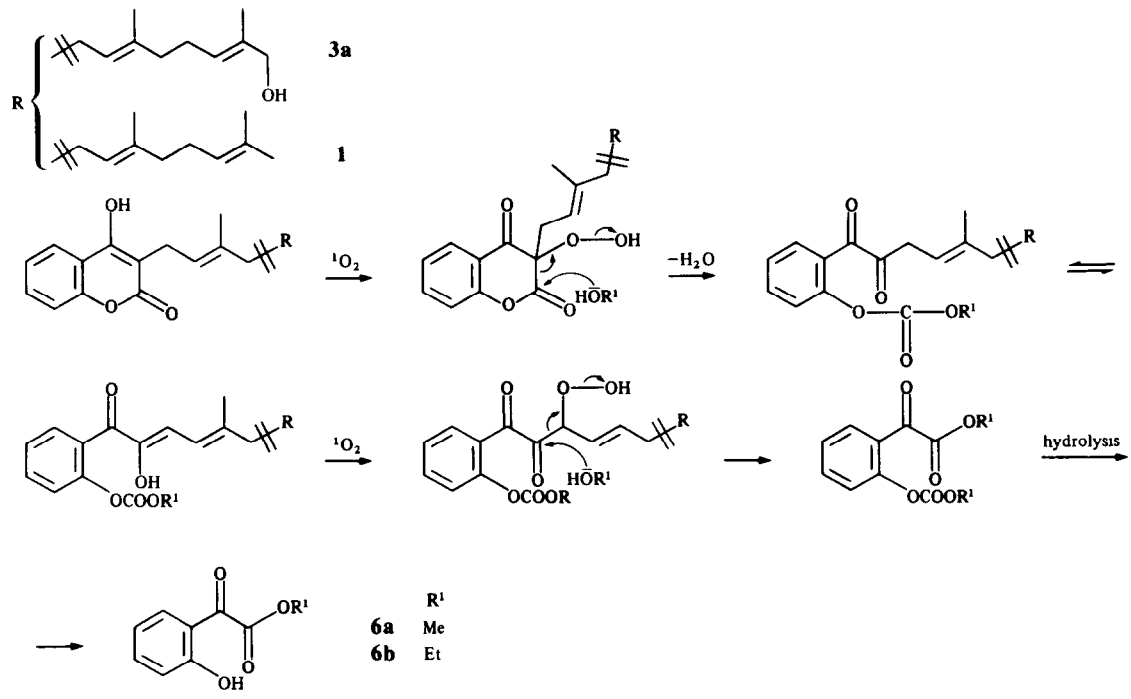
from Marocco, but it was spectroscopically characterized as its diacetate (**5b**) [9]. As regards ^{1}H NMR spectroscopy, the pairs of isomers could be distinguished on the basis of the chemical shift of the methylene bearing the oxygen function The isomer having this methylene at lower fields was assigned a *Z*-stereochemistry (**3b**, **4b**; **3d**, **4d**), and the one with this signal upfield the *E*-stereochemistry (**3a**, **4a**, **3c**, **4c**) [10] In a similar way the differences in the ^{13}C chemical shift between the pairs of isomers could be rationalized in the light of the shielding *γ-gauche* effect of C-9′ on a *cis* carbon [11] For instance, considering the pair of diastereoisomeric acetoxyferulenols (**3c** and **3d**), the ω-alkyl group resonated at higher field in **3c** (δ 13 97) than in **3d** (δ 21 25), whereas the oxygen-bearing methylene resonated at higher field in **3d** (δ 63 20) than in **3c** (δ 70.38).

The ω-hydroxy-(**3a**, **3b**) and ω-acetoxy-(**3c**, **3d**) ferulenol derivatives were chemically correlated by conversion to the same pair of 4,ω-diacetoxylated products (**5a** from **3a** and **3c**; **5b** from **3b** and **3d**) The hydroxylated heteroaromatic ring reacted with acetic anhydride exclusively in its 4-hydroxycoumarinic form, in contrast with the reaction with diazomethane, which gives also the methylderivative of the 2-hydroxychromenone tautomeric form [12, 13].

In α,β-unsaturated aldehydes bearing a β-vinyl proton, the stereochemical dependence of the chemical shift of the aldehydic methine is well established [14], and the chemical shift of the aldehydic proton in **3e** and **4e** (δ 9.38 and 9 35 respectively) showed that these products have a *E*-stereochemistry In an attempt to confirm the structure of the hydroxylated products (**3a**, **b**, **4a**, **b**), the oxidation to their respective α,β-unsaturated aldehydes was tried Oxidation with Cr^{6+} based reagent (PCC, PDC), gave exclusively the *E*-unsaturated aldehyde from each pair of isomeric alcohols (**3e** from **3a**, **b**, and **4e** from **4a**, **b**) Furthermore, in the case of the ferulenol derivatives **3a** and **3b**, the aldehyde **4e** was also obtained as a side product, resulting from the oxidative cyclization of the 3-allyl-4-hydroxycoumarinic system [8] Although this reaction is slower compared to the oxidation of the allylic hydroxyl, **4e** was also obtained when a 1 mol equivalent of reagent was used The use of MnO_2 or $BaMnO_4$ gave a complex reaction mixture, which was not further investigated Compounds **4a** and **4e** could also be obtained from the allylic oxidation of ferprenin (TBHP, SeO_2) However, this reaction was not successful with ferulenol

Compounds **3a**–**e** and **4a**–**e** were isolated from a sample of *F communis* also containing ferulenol and ferprenin, but another collection of the plant lacked both these products, as well as the (*Z*) ω-functionalized compounds, and gave instead large amounts of **3a** and **4a** Notwithstanding the absence of **1** and **2**, this sample was still highly toxic for experimental animals (M Aragno and G Ugazio, personal communication) The ω-oxygenated ferprenin derivatives isolated from both collections, have $[\alpha]_D^{25} = 0$ and are thus presumably racemates In the light of this finding, the optical rotation reported for ferprenin [8], might be due to the presence of optically active impurities, which escaped detection by HPLC or ^{1}H NMR (300 MHz)

All the ω-oxygenated compounds are unstable, and decomposition takes place in the crude extracts We investigated in detail the decomposition of **3a**. No definite compound could be obtained from its spontaneous degradation in the air Reaction with $^1O_2$ in methanol resulted instead in the smooth formation of the phenylglyoxylic ester **6a** The corresponding ethyl ester was obtained when the reaction was carried out in ethanol Ferulenol reacted in these conditions to give the same products The smooth reaction of the 4-hydroxycoumarinic nucleus with singlet oxygen is surprising, since 4-hydroxycoumarin itself is completely inert under these conditions although a reaction takes place after complexation with the fluoride anion [15] A possible mechanism for the formation of **6a** from **1** and **3a** is depicted in Scheme 1 **6b** was also isolated from exctracts of *F communis*, and it is possible that a reaction like that depicted in Scheme 1 might also take place during the manipulation of the plant material, especially if one considers that the coumarins are contained in a latex which spontaneously drips when the roots are chopped, thus resulting in the exposure of a large surface to the action of atmospheric oxygen Photooxygenation of ferprenin was a slower reaction, and gave a mixture of the isomeric hydroperoxides resulting from attack at the terminal double bond (**4f**, **4g**) These products could be



Scheme 1 Possible mechanism for the formation of **6a** and **6b** from **1** or **3a** (R′ = –Me or –Et)

separated after conversion to their corresponding alcohols (**4h**, **4i**). **4h** obtained from synthetic ferprenin was obviously a mixture of diastereoisomers, but gave only a single set of signals in the ^1H and ^{13}C NMR spectra (300 and 75.33 MHz respectively).

The toxic and the atoxic variety of *F communis* differ not only for the presence of prenylated coumarins or sesquiterpene esters, but also as regards other classes of metabolites (volatile terpenoids, phenylpropanes) We are now investigating whether these compounds might be responsible for other toxic effects of the plant, especially those on the central nervous system

EXPERIMENTAL

Silica gel 60 (70–230 mesh, Merck) was used for CC, a Water microporasyl column (80 × 3 cm) was used for prep. HPLC, using a Water 501 pump coupled with a Water differential refractometer R 401

Plant material F communis was collected at Olliena (Nu) in November 1986 (sample A) and at the Caprera island in May 1986 (sample B), and was identified by V P

Isolation of the constituents Sample A 1 7 kg of dried roots were coarsely powdered and extracted with CH_2Cl_2 (3 × 5 l) at room temp; removal of the solvent left 80 g of extract (4 7%), which was defatted by dissolving it in MeOH and cooling in the fridge for 2 weeks After removal of the ppt (fluted filter paper), the soln was evapd and partitioned between 5% Na_2CO_3 and CH_2Cl_2, to give 9.8 g of 'acidic' fraction and 65 g of 'neutral' fraction The Na_2CO_3-soluble fraction was chromatographed on a silica gel column (100 g) to give 460 mg ferulenol (crystallized from hexane), 280 mg of a mixture of **3c** and **3d** (eluent hexane–EtOAc 4 1), 600 mg of a mixture of **4a**/**4b** (eluent. hexane–EtOAc 7 3), and 5 06 g of a mixture of **3a**/**3b** (eluent hexane–EtOAc 1 1) Part of the 'neutral' fraction (8 3 g) was chromatographed on a silica gel column (100 g) to give ferprenin (98 mg after purification by HPLC), 160 mg of crude **4e**, 125 mg of a mixture of **4c**/**4d**, 160 mg of crude **4e** (eluent hexane–EtOAc 9 1), 560 mg ferulenol (crystallized from hexane), 180 mg of crude **3e**, 500 mg of a mixture of **3c**/**3d** (eluent hexane–EtOAc 4 1) The hydrophilic character of **3a** and **3b** allows their quantitative extraction with Na_2CO_3 from a CH_2Cl_2 soln of the extract. This shortens the time required for their purification with CC, and thus reduces their decomposition during the chromatographic separation Pure products were obtained using prep HPLC, the following eluents, were used purification **4e** and separation **4c**/**4d** hexane–EtOAc (9.1) purification **3e** and separation **3c**/**3d** hexane–EtOAc (9.1), separation **4a**/**4b**: hexane–EtOAc (4:1), separation **3a**/**3b**: hexane–EtOAc (7 3). Referred to the dried plant material, the yields of the purified (HPLC) products were: **3a**, 0.12%, **3b**, 0.040%; **3c**, 0 010%, **3d**, 0 040%; **3e**, 0.0090%, **4a**, 0 0060%, **4b**, 0 00090%; **4c**, 0 0020%; **4d**, 0.0010%, **4e**, 0 010%. Sample B from 1.5 kg of dried roots, 12 g of 'acidic' fraction and 98 g of 'neutral' fraction were obtained, as described for sample A. Upon CC as described for sample A, the acidic fraction gave 106 mg **6b**, 0.20 g **4a** and 6 0 g **3a** From the 'neutral' fraction (starting from 25 g of material), 82 mg **6b** and 0.40 g **4a** were obtained. The yields were: **3a**, 0 40%, **4a**, 0 15%, **6a**, 0 012% Both for sample A and sample B, the isolation of the compounds was done within 4 months after the collection of the plant The extracts from both collections could not be stored in the fridge for more than 6–7 months without extensive degradation

(E)-*ω-Hydroxyferulenol* (**3a**) Yellowish oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3300 (br), 1680, 1620, 1570, 1500, 1390, 910, 760, 735, UV λ_{EtOH}^{max} nm 312, 290, 240; EIMS 70 eV, *m/z* (rel int.) 382 214252 $[M]^+$ (calc for $C_{24}H_{30}O_4$: 382.214396) (8), 364 (18), 321 (14), 297 (20), 229 (45), 175 (100), 121 (65), ^1H NMR (270

Table 1 ^{13}C NMR data (75 33 MHz,

C	3a	3b	3c	3d	3e	4a
2	160 83 *s*	160 68 *s*	160 97 *s*	160 62 *s*	160 72 *s*	160 96 *s*
3	103 22 *s*	103 80 *s*	102 93 *s*	103 51 *s*	103 01 *s*	99 87 *s*
4	163 59 *s*	163 80 *s*	163 10 *s*	163 68 *s*	163 80 *s*	159 01 *s*
5	122 71 *d*[a]	122 69 *d*[a]	122 68 *d*[a]	122 64 *d*[a]	122 56 *d*[a]	122 64 *d*[a]
6	123 55 *d*[a]	123 67 *d*[a]	123 82 *d*[a]	123 67 *d*[a]	123 76 *d*[a]	123 36 *d*[a]
7	131 48 *d*	131 38 *d*	131 62 *d*	131 40 *d*	131 61 *d*	132 08 *d*
8	116 34 *d*	116 27 *d*	116 42 *d*	116 27 *d*	116 39 *d*	115 39 *d*
9	152 37 *s*	152.27 *s*	152 03 *s*	152 28 *s*	152 35 *s*	153 01 *s*
10	116 03 *s*	116 03 *s*	116 00 *s*	115 96 *s*	115 81 *s*	116 67 *s*
1′	23 72 *t*	23 49 *t*	23 87 *t*	23 60 *t*	23 75 *t*	117 15 *d*[d]
2′	120 16 *d*	120 34 *d*	120 08 *d*	120 03 *d*	120 11 *d*	123 94 *d*[d]
3′	141 60 *s*	140 59 *s*	142.24 *s*	141 35 *s*	141 98 *s*	83 20 *s*
4′	39 10 *t*[b]	39 59 *t*[b]	39 60 *t*[b]	39 48 *t*[b]	39 43 *t*	41 71 *t*
5′	25 74 *t*[c]	25 86 *t*[c]	25 97 *t*[c]	25 89 *t*[c]	25 80 *t*	22 40 *t*
6′	123 70 *d*	123 76 *d*	123 59 *d*	123 67 *d*	124 39 *d*	123 56 *d*
7′	135 52 *s*	135 37 *s*	135 67 *s*	135 24 *s*	134 54 *s*	135 58 *s*
8′	39 10 *t*[b]	39 35 *t*[b]	38 98 *t*[b]	39 44 *t*[b]	37 86 *t*	39 16 *t*
9′	25 74 *t*[c]	26 12 *t*[c]	25 97 *t*[c]	26 24 *t*[c]	27 23 *t*	26 09 *t*
10′	125 82 *d*	128 29 *d*	129 52 *d*	130 38 *d*	154 60 *d*	125 52 *d*
11′	134 46 *s*	134 07 *s*	129 52 *s*	129 63 *s*	139 54 *s*	134 80 *s*
12′	68 82 *t*	21 17 *q*	70 38 *t*	21 25 *q*	195 41 *d*	68 64 *t*
13′	16 31 *q*	16 31 *q*	16 43 *q*	16 27 *q*	16 30 *q*	27 50 *q*
14′	16 00 *q*	16 06 *q*	16 10 *q*	15 98 *q*	15 99 *q*	15 96 *q*
15′	13 69 *q*	61 45 *t*	13 97 *q*	63 20 *t*	9 17 *q*	13 65 *q*
OAc			170 89 *s*	170 72 *s*		
			21 02 *q*	20 80 *q*		

[a–c] Interchangeable signals

MHz, $CDCl_3$, TMS as reference) * δ 7 75 (*dd*, $J_{5,6}$ = 7 8 Hz, $J_{5,7}$ = 1 8 Hz, H-5), 7 51 (*dq*, $J_{6,7}$ = 8 5, $J_{7,8}$ = 7 8, $J_{5,7}$ = 1 8 Hz, H-7), 7 31–7 23 (*m*, H-6 + H-8), 5 40 (*m*, H-2′ + H-10′), 5 07 (*br s*, H-6′), 3 98 (*s*, H-12′), 3 38 (*d*, $J_{1',2'}$ = 7 3 Hz, H-1′), 1 80 (*br s*, H-13′), 1 61, 1 58 (*br s*, H-14′ + H-15′)

(Z)-*ω-Hydroxyferulenol* (**3b**) Yellowish oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3300 (*br*), 1675, 1620, 1570, 1500, 1460, 1385, 1220, 910, 760, 735, UV λ_{EtOH}^{max} nm 312, 290, 240, EIMS 70 eV, *m/z* (rel int) 382 214633 [M]$^+$ (calc for $C_{24}H_{30}O_4$ 382 214396) (2), 364 (5), 297 (10), 229 (50), 175 (100), 121 (60) ^{1}H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 5 36 (*br t*, $J_{9',10'}$ = 7 3 Hz, H-10′), 5.27 (*br t*, $J_{1',2'}$ = 7 3 Hz, H-2′), 5 07 (*br s*, H-6′), 4 13 (*s*, H-15′), 3 41 (*d*, $J_{1',2'}$ = 7 3 Hz, H-1′), 1 82, 1 80 (*br s*, H-12′ + H-13′), 1 59 (*br s*, H-14′)

(E)-*ω-Acetoxyferulenol* (**3c**) Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3250 (br), 1740, 1710, 1675, 1620, 1570, 1500, 1460, 1390, 1230, 760, UV λ_{EtOH}^{max} nm 312, 290, 240; EIMS 70 eV, *m/z* (rel int) 424 224747 [M]$^+$ (calc for $C_{26}H_{32}O_5$ 424 2249600) (3), 364 (40), 297 (35), 229 (55), 175 (100), 121 (95) ^{1}H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 5 40 (*m*, H-2′ + H-10′), 5 10 (*br s*, H-6′), 4 55 (*br s*, H-12′), 3 45 (*d*, $J_{1',2'}$ = 7 3 Hz, H-1′), 2 08 (*s*, OAc), 1 86 (*br s*, H-13′), 1 64, 1 63 (*br s*, H-14′ + H-15′)

(Z)-*ω-Acetoxyferulenol* (**3d**) Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3250 (br), 1740, 1710, 1675, 1615, 1575, 1500, 1460, 1390, 1230, 760, UV λ_{EtOH}^{max} nm 312, 290, 240, EIMS 70 eV, *m/z* (rel int) 424 224974 [M]$^+$ (calc for $C_{26}H_{32}O_5$ 424 224960) (5), 364 (60), 297 (62), 229 (77), 175 (100), 121 (97) ^{1}H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 5 44 (*m*, H-2′ + H-10′), 5 09 (*br s* H-6′), 4 58 (*br s*, H-15′), 3 44 (*d*, $J_{1',2'}$ = 7 3), 2 08 (*s*, OAc), 1 85 (*br s*, H-13′), 1 74 (*br s*, H-12′), 1 62 (*br s*, H-14′)

(E)-*ω-Oxoferulenol* (**3e**) Colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3300 (br), 1675, 1620, 1575, 1500, 1390, 1225, 760, UV λ_{EtOH}^{max} nm 311, 298, EIMS 70 eV, *m/z* (rel int) 380 198761 [M]$^+$ (calc for $C_{24}H_{28}O_4$ 380 198747) (3), 297 (46), 229 (58), 175 (100), 121 (85), 81 (42), ^{1}H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 9 38 (*s*, H-12′) 6 47 (*t*, $J_{9',10'}$ = 7 3 Hz, H-10), 5 41 (*t*, $J_{1',2'}$ = 7 3 Hz, H-2′), 5 12 (*br s*, H-6′), 3 43 (*d*, $J_{1',2'}$ = 7 3 Hz, H-1′), 2 46 (*q*, $J_{9',10'}$ = $J_{8',9'}$ = 7 3 Hz, H-9′), 1 85 (*br s* H-13), 1 73 (*br s*, H-15′), 1 65 (*br s*, H-14′)

(E)-*ω-Hydroxyferprenin* (**4a**) Yellowish oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3450, 1710, 1650, 1610, 1570, 1390, 1370, 1110, 1040, 920, UV λ_{EtOH}^{max} nm 375, 355, 343, 246, EIMS 70 eV, *m/z* (rel int) 380 198577 [M]$^+$ (calc for $C_{24}H_{28}O_4$ 380 198747) (18), 362 (7), 279 (100), 213 (60), 121 (46), ^{1}H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 7 80 (*dd*, $J_{5,6}$ = 7 8 Hz, $J_{5,7}$ = 1 8 Hz, H-5), 7 53 (*dq*, $J_{6,7}$, $J_{7,8}$ = 8 50 Hz, 6 7 Hz) *ca* 7 30 (*m*, H-6 + H-8), 6 59 (*d*, $J_{1,2}$ = 10 1 Hz, H-1′ or H-2′), 5 49 (*d*, $J_{1,2}$ = 10 1 Hz, H-1′ or H-2′), 5 35 (*br t*, $J_{9,10}$ = 7 3 Hz, H-10′), 5 12 (*br t*, $J_{5,6}$ = 7 3 Hz, H-6′), 3 99 (*s*, H-12′), 1 65 (*br s* H-15′), 1 57 (*br s*, H-14′), 1 53 (*s*, H-13′)

*The resonances of the aromatic protons were constants for the natural compounds of the ferulenol and ferprenin series, and are given only for **3a** and **4a**

$CDCl_3$, TMS as reference)

4d	4e	4h	4i	5a	5b
160.67 *s*	160 87 *s*	160.49 *s*	160.09 *s*	161 78 *s*	161 87 *s*
99 98 *s*	99 91 *s*	99 45 *s*	99.94 *s*	119.36 *s*	119.42 *s*
159 04 *s*	158 99 *s*	158 88 *s*	159.05 *s*	154 39 *s*	154.33 *s*
122 64 *d*[a]	122 60 *d*[a]	122 65 *d*[a]	122.64 *d*[a]	122.22 *d*[a]	122.69 *d*[a]
123 65 *d*[a]	123.74 *d*[a]	123.94 *d*[a]	123.99 *d*[a]	124.12 *d*[a]	124.29 *d*[a]
132 06*d*	132 10 *d*	132 07 *d*	132.01 *d*	131 28 *d*	131 32 *d*
115 48 *d*	115 41 *d*	116 77 *d*	116.77 *d*	116.40 *d*	116 47 *d*
153 19 *s*	153 17 *s*	153 17 *s*	153 17 *s*	151.90 *s*	151 90 *s*
116.77 *s*	116 56 *s*	117.31 *s*	115.46 *s*	116 12 *s*	116.16 *s*
117 31 *d*[d]	117 29 *d*[d]	117 30 *d*[d]	117.31 *d*[d]	24 25 *t*	24 28 *t*
125 06 *d*[d]	125 03 *d*[d]	125 03 *d*[d]	125 04 *d*[d]	118.64 *d*	118.66 *d*
83 16 *s*	83 12 *s*	83 17 *s*	83 16 *s*	137.33 *s*	137.40 *s*
41 77 *t*	41.69 *t*	41 76 *t*	41.72 *t*	39.36 *t*	39 35 *t*[b]
22 43 *t*	22 49 *t*	22 47 *t*	22.54 *t*	26.21 *t*	26.12 *t*[c]
123.96 *t*	123.75 *t*	123 94 *t*	124 09 *t*	123 95 *t*	123.90 *t*
135 32 *s*	134 36 *s*	135.70 *s*	134 69 *s*	134.31 *s*	134 24 *s*
39 48 *t*	37 84 *t*	35.55 *t*[b]	42.24 *t*	38.75 *t*	39.40 *t*[b]
26 23 *t*	27.27 *t*	33 10 *t*[b]	124 94 *d*[e]	26.11 *t*[c]	26.26 *t*[c]
123.65 *d*	154 17 *d*	75.61 *d*	139 46 *d*[e]	129 70*d*	130 21 *d*
129 04 *s*	139 37 *s*	146 98 *s*	70.66 *s*	129.16 *s*	129.51 *s*
21 40 *q*	195 17 *d*	17 58 *q*	29.82 *q*	69 92 *t*	21.14 *q*
27 54 *q*	27 61 *q*	27 40 *q*	27.60 *q*	16 07 *q*	16.11 *q*
15 97 *q*	15 88 *q*	16 00 *q*	16.03 *q*	15 72 *q*	15.74 *q*
63 20 *t*	9.20 *q*	111 05 *t*	29 82 *q*	13 63 *q*	62.94 *t*
170 80 *s*				170 65 *s*	170.72 *s*
20 97 *q*				166.53 *s*	166 59 *s*
				20.66 *q*	20 65 *q*
				20.13 *q*	20 18 *q*

(Z)-*ω-Hydroxyferprenin* (**4b**) Yellowish oil. IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3300 (br), 1700, 1645, 1610, 1570, 1500, 1460, 1370, 760; UV λ_{EtOH}^{max} nm 375, 355, 343, 245, EIMS 70 eV, *m/z* (rel int.): 380 198577 $[M]^+$ (calc for $C_{24}H_{28}O_4$ 380 198747) (14), 279 (100), 213 (50), 121 (50), ^1H NMR (270 MHz, $CDCl_3$, TMS as reference): δ 5 24 (*br t*, $J_{9',10'}$ = 7 3, H-10'), 5.11 (*br t*, $J_{5',6'}$ = 7.3 Hz, H-6'), 4 10 (*s*, H-15'), 1 78 (*br s*, H-12'), 1.54 (*br s*, H-14'), 1.52 (*s*, H-13').

(E)-*ω-Acetoxyferprenin* (**4c**) Yellowish oil IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1720, 1640, 1610, 1570, 1560, 1230, 1040. UV λ_{EtOH}^{max} nm 375, 355, 343, 245, EIMS 70 eV, *m/z* (rel. int) 422 $[M]^+$ $(C_{26}H_{30}O_5)^+$ (5), 362 (8), 213 (100); ^1H NMR (300 MHz, $CDCl_3$, TMS as reference): δ 5.40 (*t*, $J_{9',10'}$ = 7 3 Hz, H-10'), 5.11 (*br t*, $J_{5',6'}$ = 7 3 Hz, H-6'), 4 43 (*s*, H-12'), 2.07 (OAc), 1 63 (*br s*, H-15'), 1 56 (*br s*, H-14'), 1 52 (*s*, H-13')

(Z)-*ω-Acetoxyferprenin* (**4d**) Yellowish oil. IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1720, 1640, 1610, 1570, 1490, 1370, 1230, 1040, UV λ_{EtOH}^{max} nm: 375, 355, 343, 245, EIMS 70 eV, *m/z* (rel. int.) 422 $[M]^+$ $(C_{26}H_{30}O_5)^+$ (2), 362 (8), 213 (100), ^1H NMR (300 MHz, $CDCl_3$, TMS as reference) δ 5.33 (*t*, $J_{9'10'}$ = 7 3 Hz, H-10'), 5 11 (*br t*, $J_{5',6'}$ = 7 3 Hz, H-6'), 4 55 (*s*, H-15'), 2 07 (OAc), 1 72 (*br s*, H-12'), 1.54 (*br s*, H-14'), 1 52 (*s*, H-13')

(E)-*ω-Oxoferprenin* (**4e**). Yellowish oil. IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1710, 1690, 1640, 1610, 1520, 1490, 1420, 1365, 1120, 1040, 760; UV λ_{EtOH}^{max} cm^{-1} 375, 355, 320, 306, 281, EIMS 70 eV, *m/z* (rel. int.) 378. 183197 $[M]^+$ (calc for $C_{24}H_{26}O_4$: 378 183098) (37), 363 (12), 279 (90), 213 (100), 121 (95), ^1H NMR (300 MHz, $CDCl_3$, TMS as reference) δ 9 35 (*s*, H-12'), 6 41 (*t*, $J_{9',10'}$ = 7.3 Hz, H-10'), 5 12 (*br t*, $J_{5',6'}$ = 7 3 Hz, H-6'), 1.75 (*br s*, H-15'), 1 57 (*br s*, H-14'), 1 52 (*s*, H-13').

Acetylation of **3a** A 250 mg sample of **3a** (0.65 mmol) was dissolved in 1 ml pyridine (20 mol equiv.) and treated with 1 24 ml Ac_2O (20 mol equiv.). The soln. was stirred overnight at room temp. under N_2, and then worked-up by the addition of ice and a few drops of MeOH. When all the ice had melted, CH_2Cl_2 was added, and the organic phase was separated, washed with dil. HCl, sat $NaHCO_3$, satd $CuSO_4$ and brine After drying $MgSO_4$) and removal of the solvent, the residue was purified by prep. HPLC (hexane–EtOAc 4.1) to give 171 mg **5a** as a colourless oil (yield: 57%). IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1780, 1730, 1640, 1620, 1360, 1240, 1170, 1090, 760; UV λ_{EtOH}^{max} nm: 320, 310, 271, EIMS 70 eV, *m/z* (rel. int): no molecular ion, 406 $[M-60]^+$ (2), 346 (3), 175 (70), 121 (100). ^1H NMR (300 MHz, $CDCl_3$, TMS as reference): δ 7.52–7.24 (*4H*, aromatic protons), 5 41 (*br t*, $J_{9',10'}$ = 7.3 Hz, H-10'), 5 17 (*br t*, $J_{1',2'}$ = 7.3 Hz, H-2'), 5.09 (*br t*, $J_{5',6'}$ = 7.3 Hz, H-6'), 4 43 (*s*, H-12'), 3 23 (*d*, $J_{1',2'}$ = 7 3 Hz, H-1'), 2.45 (*s*, OAc), 2.07 (*s*, OAc), 1.76 (*br s*, H-13'), 1.63 (*br s*, H-15'), 1.58 (*br s*, H-14')

Acetylation of **3b**, **3c** *and* **3d**. The same procedure used for **3a** was employed, but in the case of **3c** and **3d**, only 10 mol equiv of pyridine and Ac_2O were employed The reaction mixtures were purified by HPLC as described for the acetate of **3a**. The diacetates **5b** (from **3b** and **3d**) and **5a** (from **3c**) were obtained **5b** was a colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1780, 1730, 1640, 1610, 1460, 1360, 1245, 1170, 760; UV λ_{EtOH}^{max} nm. 320, 310, 271, EIMS 70 eV, *m/z* (rel int.): 466 $[M]^+$ (< 1), 346 (< 1), 175 (60); 121 (100), ^1H NMR (300 MHz, $CDCl_3$, TMS as reference). δ 7 52–7.24 (4H, aromatic protons), 5.43 *br t*, $J_{9',10'}$ = 7 3 Hz, H-10'), 5.17 (*br t*, $J_{1',2'}$ = 7.1 Hz, H-2'), 5.07 (*br s*, H-6'), 4.55 (*s*, H-15'), 3 23 (*d*, $J_{1',2'}$ = 7.1 Hz, H-1'), 2 45 (*s*, OAc), 2 06 (*s*, OAc), 1 75 (*br s*, H-13'), 1 72 (*br s* H-12'), 1.56 (*br s*, H-14')

Oxidation of **3a**. A 200 mg sample of **3a** (0.68 mmol) in 1.5 ml

CH_2Cl_2 was added to a stirred suspension of PCC (218 mg, 1 02 mmol, 1 5 mol equiv) in 1 5 ml CH_2Cl_2 After 10 min, all **3a** had reacted, and the reaction was worked-up by the addition of celite® and Et_2O After filtration through a short pad of silica gel, the residue was purified by HPLC (hexane–EtOAc 4 1) to give 42 mg **3e** (yield 21%) and 21 mg **4e** (yield 11%)

Oxidation of **3b**, **4a** *and* **4b**. The same procedure used for **3a** was employed The reaction with **3b** and **4b** was slower, and required *ca* 50 min for the complete disappearance of the starting material After purification by HPLC (same conditions used for the separation for the reaction mixture from the oxidation of **3a**), the aldehydes **3e** (25% from **3b**) and **4e** (18% from **3b**, 52% from **4a** and 40% from **4b**) were obtained

Photooxygenation of **3a** To a soln of **3a** (138 mg) in 5 ml MeOH, 2 5 mg Methylene Blue were added, and the soln was stirred under an O_2 atmosphere and irradiated with an halogen lamp (500 W) with stirring and cooling The course of the reaction was followed by TLC (hexane–EtOAc 3 2 R_f **3a** 0 21, R_f **6a** 0 55) After 4 hr, all **3a** had reacted, and the reaction mixture was worked-up by filtration through a short pad of silica gel to remove the dye, and evapd The residue was purified by CC (5 g silica gel, hexane–EtOAc 4 1) 28 mg of **6a** were obtained (yield 43%) as a yellowish oil IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3600 2800 (*br*), 1745, 1640, 1580, 1490, 1460, 1210, 1160, 1010, 760, EIMS 70 eV, *m/z* (rel int) 180 $[M]^+$ $[C_9H_6O_4]^+$ (13), 121 $[M-59]^+$ (100), 111 (45), 93 (53), 69 (30), 55 (20), 43 (54), ^1H NMR (270 MHz, $CDCl_3$, TMS as reference) δ 11 17 (s, OH), 7 71 (*dd*, $J_{5,6}-8\ 0$ Hz, $J_{5,7}=1\ 8$ Hz, H-6), 7 57 (*dq*, $J_{4,5}=8\ 3$ Hz, $J_{3,4}=7\ 8$ Hz, $J_{4,6}-1\ 8$ Hz, H-4), 7 04 (*d*, $J_{3,4}=8\ 3$ Hz, H-3), 6 97 (*dd*, $J_{5,6}=8\ 0$ Hz, $J_{4,5}=8\ 3$ Hz, H-5), 4 01 (OMe) The reaction carried out in EtOH under the same conditions, gave 29 mg **6b** from 110 mg **3a** (yield 52%) (R_f **6b** 0 58, hexane–EtOAc 3 2) The reaction could be carried out also using a desk-table lamp, in this case the disappearance of the starting material was slower, and the reaction required *ca* 8 hr for completion

6b was a yellowish oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3600–2800 (*br*), 1745, 1640, 1200, 1160, 1030, 760, EIMS 70 eV, *m/z* (rel int) 194 $[M]^+$ $[C_{10}H_{10}O_4]^+$ (51), 121 $[M-73]^+$ (100), 93 (48), 65 (51), 53 (86), 39 (37), ^1H NMR (270 MHz, $CDCl_3$, TMS as reference) aromatic protons and -OH identical to (**6a**), δ4 48 (*q*, $J=7$ Hz), 1 14 (*t*, $J=7$ Hz) (OEt), ^{13}C NMR (67 80 MHz, $CDCl_3$, TMS as reference) δ190 75 *s*, 163 65 *s*, 162 39 *s*, 138 02 *d*, 132 03 *d*, 119 55 *d*, 118 44 *d*, 116 03 *s*, 29 57 *t*, 13 95 *q* The IR, ^1H- and ^{13}C NMR spectra of **6b** isolated from the extract, were identical to those of **6b** obtained from the photooxygenation of **3a** **6a** and **6b** could also be obtained in comparable yields when ferulenol was photooxygenated in these conditions (hexane–EtOAc 4 1 was used to follow the reaction by TLC The R_f values were ferulenol 0 19, **6a** 0 35, **6b** 0 39) When CH_2Cl_2 was used as the solvent for the reaction, the disappearance of ferulenol was faster (*ca* 2 hr, desk table lamp) From the reaction mixture, no definite compound could be isolated

Photooxygenation of ferprenin A 238 mg sample of ferprenin was dissolved in 6 ml MeOH containing 5 mg Methylene Blue The mixture was stirred with cooling under an O_2 atmosphere and irradiated with a halogen lamp (500 W) After 24 hr, the reaction mixture was worked-up by filtration of the dye on a short pad of silica gel and by evapn The residue was chromatographed on a silica gel column (20 g), eluted with hexane–EtOAc (4 1), to give 40 mg of unreacted ferprenin and 127 mg of a mixture of the hydroperoxides **4f** and **4g** A 80 mg mixture of these compounds was dissolved in 0 5 ml CH_2Cl_2, and treated with 53 mg (1 0 mol equiv) of TPP After 5 min the soln was chromatographed on a silica gel column (5 g) eluted with hexane–EtOAc (7 3), to give 43 mg **4h** and 23 mg **4i** (yield 27% and 15% respectively) **4h** was a colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3450, 3070, 1710, 1645, 1610, 1570, 1495, 1420, 1370, 1115, 1040, 915, 760, 730, UV λ_{EtOH}^{max} nm 375, 355, 343, 246 EIMS 70 eV, *m/z* (rel int) 380 $[M]^+$ $[C_{24}H_{28}O_4]^+$ (5), 213 (100), ^1H NMR (300 MHz $CDCl_3$, TMS as reference) δ 5 15 (*br t*, $J_{5',6'}=7\ 3$ Hz, H-6'), 4 91 (*br s*, H-15'a) 4 82 (*br s*, H-15'b), 4 00 (*t*, $J_{9',10'}=6\ 6$ Hz, H-10'), 2 15 (*q*, $J_{5',6'}=J_{4',5'}=7\ 3$ Hz) 1 70 (*br s*, H-12'), 1 57 (*br s*, H-14'), 1 52 (*s*, H-13') **4i** was a colourless oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1} 3450, 1710, 1645, 1610 1570, 1490 1420, 1365, 1110, 1040, 975, 915, 760, 730, UV λ_{EtOH}^{max} nm 375, 355, 343, 246, EIMS 70 eV, *m/z* (rel int) 380 $[M]^+$ $[C_{24}H_{28}O_4]^+$(3) 213 (100), ^1H NMR (300 MHz, $CDCl_3$, TMS as reference), δ 5 60 (*d*, $J_{9',10'}=15\ 6$ Hz, H-10'), 5 54 (*m*, H-9'), 5 12 (*br t*, $J_{5',6'}=7\ 3$ Hz, H-6'), 2 60 (*d*, $J_{8',9'}=5\ 9$ Hz, H-8'), 2 15 (*q*, $J_{4',5'}=J_{5',6'}=7\ 3$ Hz, H-5'), 1 52 (*s*, H-13'), 1 30 (*s*, H-14' + H-15')

Allylic oxidation of ferprenin TBHP (1 02 ml, 70% aq soln , 7 4 mmol, 2 mol equiv) was added to a stirred suspension of SeO_2 (205 mg, 1 8 mmol, 0 5 mol equiv) in CH_2Cl_2 (5 ml) When all the SeO_2 had dissolved, the reaction was cooled to 0°, and 1 35 g ferprenin (3 7 mmol) dissolved in 5 ml CH_2Cl_2 added dropwise After stirring for 7 hr at room temp , the reaction was worked-up by the addition of a 10% Na_2SO_3 soln After stirring for 10 min at room temp , the phases were separated, and the organic layer was washed with brine and dried ($MgSO_4$) After removal of the solvent, the residue was separated by CC (10 g silica gel, hexane–EtOAc 4 1), to give 109 mg of unreacted ferprenin, 83 mg of **4e** (6% yield) and 351 mg **4a** (yield 25%) **4a** and **4c** obtained from the oxidation of ferprenin were identical (IR, ^1H NMR) with the corresponding natural products isolated from *F communis*

Acknowledgements This work was supported by the Ministero della Pubblica Istruzione (fondi 40 and 60%) We are grateful to Prof C Bicchi and C Frattini for the mass spectra, and to Dr M G Valle for help in the early part of this research

REFERENCES

1 Moris, J (1840) *Flora Sardoa*, Vol 2, p 1
2 Altara, I (1925) *Nuova Vet* **3**, 194
3 Crovetti, A (1963) *Studi Sassaresi, Sect III*, **XI** 651
4 Bremond, R (1887) *Journal Veterinaire de l'Ecole de Lyon* (quoted in ref 3)
5 Carta, A (1949) *Atti Soc Ital Scienze Vet* **III**, 239
6 Valle, M G , Appendino, G , Nano, G M and Picci, V (1987) *Phytochemistry* **26** 253
7 Arango, M , Ugazio, G , Nano G M and Tagliapietra, S (1988) *Res Commun Chem Pathol Pharmacol* **59**, 399
8 Appendino, G , Tagliapietra S Nano, G M and Picci V (1988) *Phytochemistry* **27**, 944
9 Lamnaouer, D , Bodo, B , Martin, M T and Molho, D (1987) *Phytochemistry* **26**, 1613
10 Corey, E J and Yamamoto, H (1970) *J Am Chem Soc* **92**, 226
11 De Haan, J W and van de Ven L J M (1973) *Org Magn Res* **5**, 147
12 De Pasqual Teresa, J , Villaseco, M A , Hernández, J M , Moran, J R Urones J C and Grande, M (1986) *Planta Med* 458
13 Ellis, G P (1972) in *The Chemistry of Heterocyclic Compounds* (Weissberger, A and Taylor, E C eds) Vol 31 (Ellis, G P ed) pp 489–492 Wiley, New York
14 Chan, K C , Jewell, R A Nutting, W H and Rapoport, H (1968) *J Org Chem* **33** 3382
15 Wasserman, H H and Pickett J F (1985) *Tetrahedron* **41**, 2155