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ACETOGENINS FROM *PORCELIA MACROCARPA*: STEREOCHEMICAL DETERMINATION OF 2-ALKYL-3-HYDROXY-4-METHYL γ-LACTONES BY ^{13}C NMR SPECTROSCOPY*

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Key Word Index—*Porcelia macrocarpa*; Annonaceae; Acetogenins; acetylenic compounds; ^{13}C NMR spectroscopy.

Abstract—Seeds of *Porcelia macrocarpa* afforded two acetogenins: (2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(9-*n*-eicos-1-enyl) butanolide and the corresponding 9-*n*-eicosanyl derivative. Their absolute stereochemistries were determined by chemical ^{1}H and ^{13}C NMR and mass spectroscopic methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In continuation of our studies on the Brazilian Annonaceae [1], we analysed the native species *Porcelia macrocarpa* (Warming) R. E. Fries. Previous studies on this small genus *Porcelia* Ruiz & Pavón (seven species) [2] focused on the constituents of the non-saponifiable fraction of *P. macrocarpa* foliar epicuticular wax [3]. In this study, the chemical constituents of the dichloromethane extract of the green fruit seeds of *P. macrocarpa* were examined, and found to contain various acetogenins, triglycerides and fatty acids.

RESULTS AND DISCUSSION

The dichloromethane extract of the green fruit seeds of *P. macrocarpa* was fractionated by silica gel column chromatography to give a mixture of substituted butyrolactones (**1** and **2**), as well as triglyceride and fatty acid fractions. Subsequent separation by RP-18 HPLC afforded pure **1** and **2**. The ^{1}H and ^{13}C NMR spectra of each compound established that they were γ-lactone containing acetogenins differing only in the level of unsaturation at the side-chain terminus.

Compound **1** was hydrogenated to give the saturated chain γ-lactone **1a**. GC-mass spectroscopy showed that **1a** was a mixture of $C_{20}H_{41}$ and $C_{16}H_{33}$ side-chain lactones, with the former being predominant. The COSY ^{1}H–^{1}H and ^{1}H–^{13}C NMR spectra (Table 1) were used to determine the constitution of the γ-lactone ring as A (Fig. 1).

At this point two aspects needed to be established: namely, the stereochemistry of the lactone ring and the position of the triple bond in the chain. It is well known that coupling constants observed in the ^{1}H NMR spectra of five membered ring compounds do not give conclusive information about the relative configuration of the substituents on the ring. The all-*cis* stereochemistry of the lactone ring substituents of **1** and **2** was determined as follows. First, an intense nuclear Overhauser effect (NOE) signal between H-2 and H-4 was observed, suggesting a *cis* quasi-axial relationship between them. (NOEs between other protons were also noted but they were not conclusive due to overlapping NMR signals.) Assuming this quasi-axial relationship, the methine proton at C-3 may be either *cis* or *trans* to H-2 and H-4, but analysis of the coupling constants J_{H2-H3} (3 Hz) and J_{H3-H4} (5 Hz) suggested a *cis* relationship for all hydrogen atoms (vicinal angles around 40°) [4]. Finally, analysis of the ^{13}C NMR spectroscopic data of the four possible relative configurations for A agree with the suggested configuration (E) for **1** and **2** (Fig. 1). It is clear that when the methyl is *cis* to the hydroxyl group, its ^{13}C chemical shift is approximately δ 13, and when the relative configuration is *trans* it is approximately δ 18 ppm. The same observation could be deduced from the chemical shift of CH_2 at C-2: when it is *cis* to the hydroxyl group, the signal appears at approximately δ 23, but when it is *trans* the carbon is deshielded and the signal appears at about δ 27 (Table 2). These observations are conclu-

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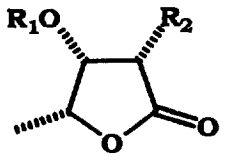


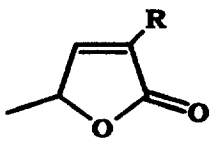
1 R_1 = H; R_2 =

2 R_1 = H; R_2 =

1a R_1 = H; R_2 = $C_{20}H_{41}$

1b R_1 = Ac; R_2 =

2b R_1 = Ac; R_2 =



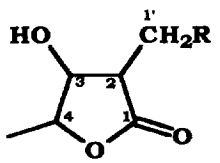
1c R =

2c R =

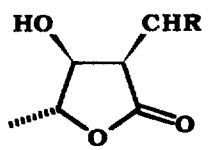
sive to determine the relative stereochemistry of all lactone rings with configuration A.

The $[\alpha]_D^{25}$ values of **1** (+39.4°, MeOH) and of the mixture **1** + **2** (+41.2, MeOH) prove that both **1** and **2** have the same configuration. The dehydration product of the mixture (**1** + **2**) gave $[\alpha]_D^{25} = -31.5°$ dioxane. Comparison of these values with the $[\alpha]_D^{25}$ of a similar compound [10] showed that C-4 has configuration (*R*), and consequently C-3 and C-2 are (*R*) and (*S*), respectively.

The position of the triple bond in the methylenic side-chain was first suggested by the comparative mass



A



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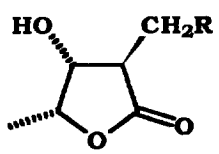

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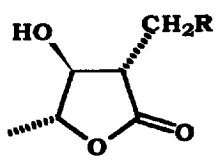
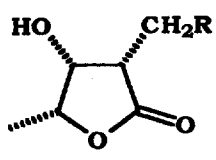


D



E

Fig. 1. Relative configurations of the 2-alkyl-3-hydroxy-4-methyl butanolides.

Table 1. NMR spectroscopic data of natural acetogenins and their derivatives ($CDCl_3$, δ, TMS)

Carbon no.	δH*				δC†			
	1	**2**	**1b**	**1c**	**1**	**2**	**1b**	**1c**
1	–	–	–	–	178.1	177.9	176.3	173.8
2	2.57 *dt* (5; 9.7)	2.57 *dt* (5; 9.2)	2.52 *dt* (5.2; 9.2)	–	47.5	47.6	45.6	134.3
3	4.31 *dd* (5; 3)	4.30 *dd* (5; 3)	5.43 *dd* (5.2; 3.1)	6.96 *d* (1.5)	71.0	71.2	72.3	148.8
4	4.46 *dq* (3; 6.5)	4.45 *dq* (3; 6.5)	4.42 *dq* (3.1; 5.8)	4.95 *m*	79.1	79.0	77.2	77.4
Me-4	1.43 *d* (6.5)	1.44 *d* (6.6)	1.29 *d* (5.8)	1.38 *d* (6.8)	13.6	13.7	13.9	19.2
1′	1.72 *m*	1.69 *m*	1.70 *m*	2.24 *t* (7)	23.2	23.3	23.6	25.2
2′	1.28 *br s*	1.28 *br s*	1.23 *br s*	1.47 *m*	27.5	27.6	27.2	27.4
3′–9′	1.28 *br s*	1.28 *br s*	1.23 *br s*	1.25 *br s*	28.5–29.4	28.5–29.6	28.6–29.6	28.6–29.7
10′	2.13 *t* (6.8)	2.13 *t* (6.8)	2.10 *t* (6.5)	2.11 *t* (6.4)	18.7	18.7	18.7	18.7
11′	–	–	–	–	80.2	80.2	80.2	80.2
12′	–	–	–	–	80.2	80.2	80.1	80.1
13′	2.13 *t* (6.8)	2.13 *t* (6.8)	2.10 *t* (6.5)	2.11 *t* (6.4)	18.7	18.7	18.7	18.7
14–17′	1.28 *br s*	1.28 *br s*	1.23 *br s*	1.25 *br s*	28.5–29.4	28.6–29.6	28.6–29.6	28.6–29.7
18′	2.02 *t* (6.6)	1.28 *br s*	1.99 *t* (6.6)	2.00 *t* (6.6)	33.7	31.8	33.6	33.6
19′	5.81 *ddt* (17; 10; 6.6)	1.28 *br s*	5.62 *ddt* (17; 10; 6.6)	5.77 *ddt* (17; 10; 6.6)	139.0	22.6	139.0	139.1
20′	4.97 *m*	0.88 *t* (6)	4.78 *m*	4.94 *m*	114.1	14.1	114.1	114.1
Ac	–	–	2.10 *s*	–	–	–	20.3; 169.9	–

*200 MHz.

†50, 3 MHz.

Table 2. ^{13}C NMR data for butanolides with relative configurations B, C, D and E

C	B* [5–7]	C [6, 8]	D* [9]	E
2	43.7	49.2	48.4	47.5
3	73.8	74.1	80.1†	71.2
4	82.3	78.0	78.7†	79.0
Me-4	18.0	13.8	18.1	13.7
1′	23.3	27.2	–‡	23.3

*The stereochemistry proposed elsewhere [5–7] does not agree with our analysis.

†The signal could be interchanged.

‡The value was not published, probably due to the overlapping of the $-(CH_2)_n^-$ signal.

spectroscopic analyses of **1**, **2**, **1b** and **1c**. The spectra showed the same overall pattern, indicating that the triple bond of the acetogenins is at C-11′ [11] (Fig. 2). The mass spectrum of the ketal products of **2**, formed by the reaction with ethylene glycols and Hennion catalyst [12], presents two pairs of ions (*m/z* 199, 309 and 185, 323) originating from the fragmentation of **2d** and **2e**, respectively, that agree with the suggested structures of **1** and **2** (Fig. 3).

The biogenesis of **1** and **2** involves a Claisen condensation of a C_{22} fatty acid with pyruvic acid [9]. The triple-bond position was expected to be on C-9 of the fatty acid, instead of C-13 as in **1** and **2**. In this case, the C_{18} acid was probably first dehydrogenated and then condensed with two more malonyl SCoA units to afford the C-22 acid with unsaturation at C-13.

EXPERIMENTAL

General. Mp: uncorr. ^1H 200 MHz and ^{13}C 50.3 MHz NMR spectra were recorded with TMS as int. standard. EI-MS: 70 eV. Silica gel, 63–210 μm was used for CC. Semi-prep. HPLC: Perkin-Elmer series 3B (ODS 2.2 cm i.d. × 25 cm) using MeOH–H_2O as solvent and UV detector operated at 230 nm.

Plant material. Green fruits of *P. macrocarpa* (Warm.) R. E. Fries were collected at the Jardim Botânico of São Paulo, June 1991. A voucher specimen has been deposited in the Herbaria of the Instituto Botânico, São Paulo, Brazil under reference SP76791.

Extraction and isolation of compounds. Dried and ground seeds of the green fruits of *P. macrocarpa* (146 g) were defatted with petrol and the residue was then extracted with CH_2Cl_2 (2 × 500 ml). The CH_2Cl_2 extracts were then combined and evaporated *in vacuo* to give a crude extract (11 g). The CH_2Cl_2 extract was fractionated by CC on silica gel using a petrol–EtOAc gradient. Petrol–EtOAc (9:1) eluate gave triglycerides (4 g). Petrol–EtOAc (8:2) eluate gave **1** + **2** (2 g); petrol–EtOAc (7:3) eluate gave fatty acid mixtures (3 g). Prep. RP-18 HPLC of the mixture of **1** and **2** (400 mg), eluted with MeOH–H_2O (75:35) afforded pure **1** (240 mg) and **2** (63 mg).

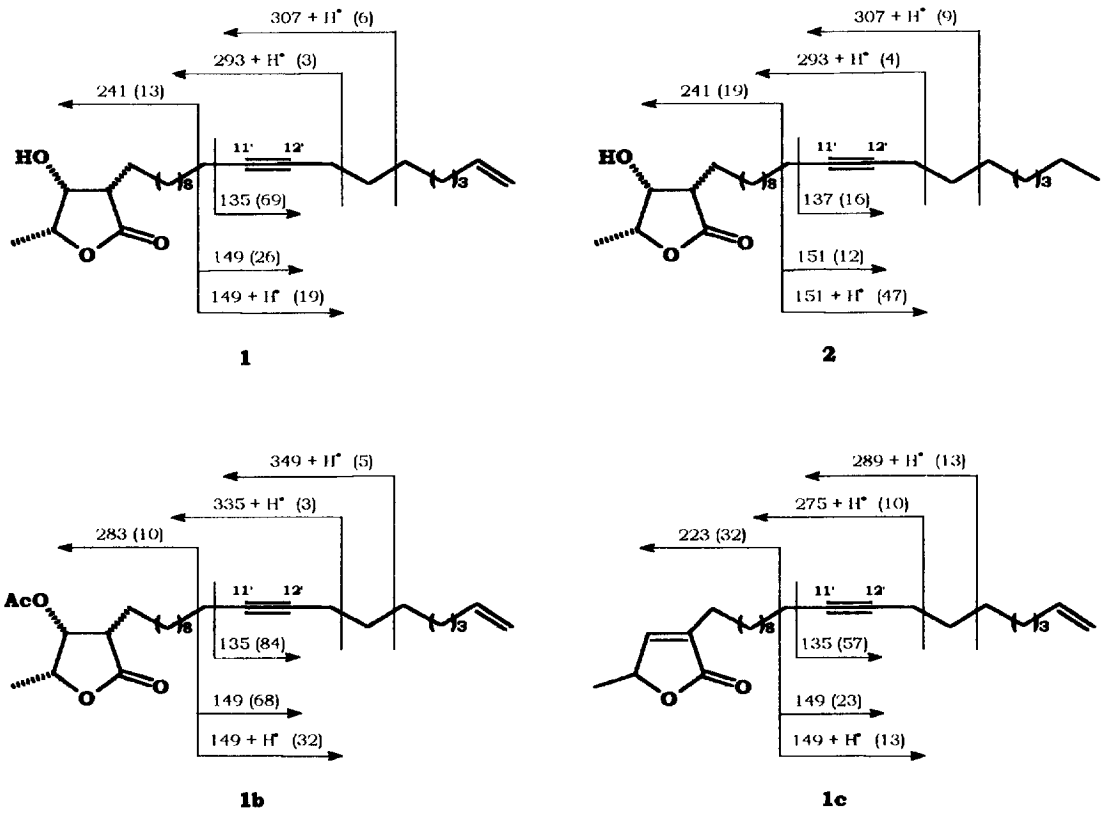


Fig. 2. Mass spectrometric acetylenic fragments of compounds **1**, **2**, **1b** and **1c**.

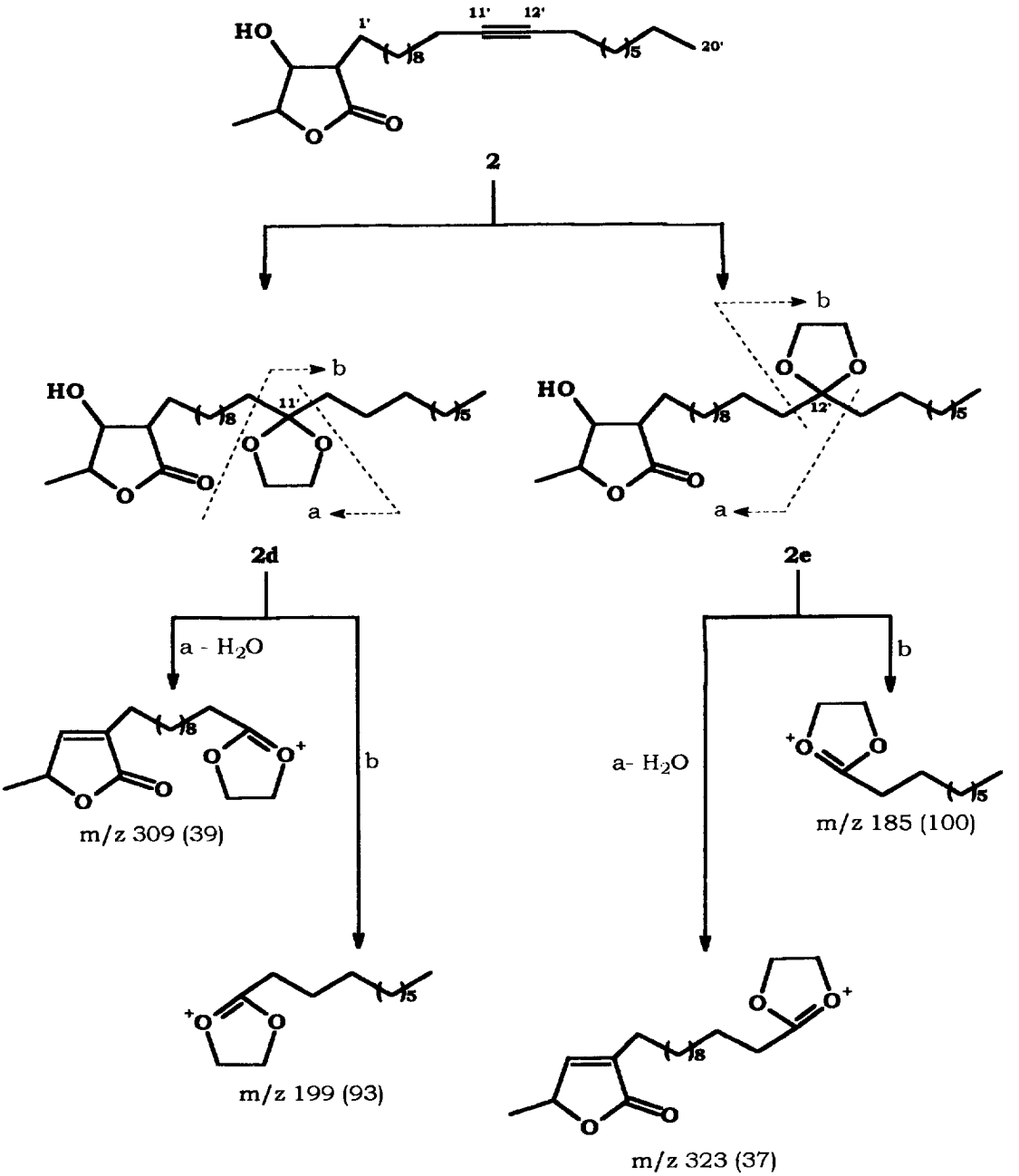


Fig. 3. Mass spectrometric ketal fragments of compounds **2d** and **2e**.

(2S,3R,4R)-3-*Hydroxy*-4-*methyl*-2-(9-n-*eicos*-1-*enyl*) *butanolide* (**1**). Crystals, MP 78.0–78.2° (MeOH). $[\alpha]_D^{25}$ +39.4° (MeOH; *c* 0.33). IR (ν_{max}^{KBr} cm^{-1}): 3415, 2984, 2920, 2852, 1760, 1741, 1643, 1469. EIMS 70 eV, *m/z* (rel. int.): 390 $[M]^+$ (1), 308 (6), 294 (3), 241 (13), 227 (7), 185 (6), 177 (7), 171 (5), 164 (8), 163 (10), 150 (19), 149 (26), 136 (21), 135 (69), 129 (25), 123 (14), 122 (23), 121 (48), 116 (21), 111 (14), 99 (20), 95 (64), 94 (47), 81 (71), 80 (48), 69 (54), 68 (33), 67 (73), 57 (100), 55 (67), 41 (62). ^1H and ^{13}C NMR: see Table 1.

(2S,3R,4R)-3-*Hydroxy*-4-*methyl*-2-(9-n-*eicosanyl*) *butanolide* (**2**). Amorphous powder. IR (ν_{max}^{film} cm^{-1}): 3421, 2922, 2852, 1760, 1742, 1469. EI-MS 70 eV, *m/z* (rel. int.): 392 $[M]^+$ (2), 308 (9), 294 (4), 241 (19), 227 (10), 185 (10), 177 (4), 171 (6), 167 (14), 166 (20), 152 (47), 151 (12), 137 (16), 136 (16), 135 (26), 129 (29), 123 (23), 122 (12), 121 (22), 116 (27), 111 (18), 99 (24), 95 (74), 94 (25), 81 (77), 80 (28), 69 (36), 68 (44), 67 (72), 57 (100), 55 (70), 41 (57). ^1H and ^{13}C NMR: see Table 1.

Catalytic hydrogenation of **1**. Acetogenin **1** (72 mg) dissolved in $CHCl_3$ (6 ml) was added to a prehydrogenated suspension of 10% Pd-C (36 mg) in $CHCl_3$ (5 ml) and hydrogenated at room temp. for 4 hr. The soln was filtered and evapd to give **1a** (63 mg). IR

(ν_{max}^{KBr} cm^{-1}): 3433, 2920, 2849, 1732, 1470. GC-EI-MS 70 eV, *m/z* (rel. int): 396 $[M]^+$ (1), 129 (86), 116 (100), 111 (29), 99 (37), 81 (13), 79 (4), 67 (13), 57 (91), 55 (34), 41 (36).

Acetylation of **1**. Acetogenin **1** (20 mg) dissolved in pyridine (1 ml) was treated with Ac_2O (1 ml) and left overnight at room temp. After the usual procedure, acetate **1b** (21 mg) was obtained as an oil. IR (ν_{max}^{film} cm^{-1}): 2929, 2856, 1783, 1749, 1641; 1462, 1229. EI-MS 70 eV, *m/z* (rel. int.): 433 (15), 432 $[M]^+$ (5), 350 (5), 336 (3), 283 (10), 205 (7), 191 (10), 177 (17), 171 (8), 167 (17), 153 (8), 150 (32), 149 (68), 136 (31), 135 (84), 129 (9), 123 (23), 122 (35), 121 (68), 116 (5), 111 (14), 109 (49), 108 (45), 107 (55), 99 (50), 95 (78), 94 (62), 93 (65), 81 (88), 80 (64), 79 (72), 69 (56), 68 (53), 67 (97), 57 (89), 55 (100). ^1H and ^{13}C NMR: see Table 1.

Elimination of HOAc from **1b**. Compound **1b** (20 mg) was placed on the top of an Al_2O_3 90 (Merck activity II/III, 6 g) column. Elution with pentane gave **1c** (15 mg). IR (ν_{max}^{film} cm^{-1}): 2929, 2855, 1759, 1642, 1461. EIMS 70 eV, *m/z* (rel. int.): 372 $[M]^+$ (5), 290 (13), 276 (10), 237 (6), 223 (32), 209 (20), 205 (4), 195 (6), 191 (6), 177 (10), 167 (9), 164 (9), 163 (10), 153 (10), 150 (13), 149 (23), 136 (17), 135 (57), 123 (19), 122 (19), 121 (41), 111 (17), 109 (37), 108 (23), 107 (35) 95 (73), 99 (3), 94 (36), 93 (51), 81 (87), 80 (35), 79 (69), 69 (32), 68 (30), 67 (100), 57 (11), 55 (81). ^1H and ^{13}C NMR: see Table 1.

Acetylation of **1** *and* **2**. Mixture of **1** and **2** (31 mg) was acetylated by the same procedure described for **1** to give **1** + **2** acetate (**1b** + **2b**) (33 mg).

Elimination of HOAc from **1b** + **2b**. The elimination of HOAc of **1b** + **2b** (32 mg) was done by the same procedure described for **1b**. A mixture of **1c** and **2c** (25 mg) was obtained, $[\alpha]_D^{25}$ −31° (dioxane, approx. 2.38).

Reaction of **2** *with ethylene glycol*. Acetogenin **2** (40 mg) dissolved in ethylene glycol (10 ml) was heated at 60–70° for 1 hr with a trace of Hennion's catalyst [12]. The crude product was taken up in ether, washed with sodium carbonate solution, dried over sodium sulphate and the solvent removed under red. pres. The crude mixture analysed by GC-MS showed the presence of two ethylene ketals (**2d** and **2e**). MS: see Fig. 2.

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