Journal of Chromatography A, 658 (1994) 123–127
Elsevier Science B.V., Amsterdam

CHROMSYMP. 2888

Chromatographic analysis of G_1–G_3 natural cyclic peroxides and compounds obtained from Ru(II)-catalysed reaction of G_3

M. Baltas, M. Benbakkar, L. Gorrichon* and C. Zedde

Laboratoire de Synthèse et Physicochimie Organique Associé au CNRS, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse (France)

ABSTRACT

Compounds obtained in the Ru(II)-catalysed reaction of endoperoxide G_3 were analysed by means of liquid and capillary gas chromatography. The chromatographic data obtained by analytical liquid and capillary gas chromatography of the natural endoperoxides G_1, G_2, G_3 are discussed.

INTRODUCTION

Natural peroxides constitute important biological mediators in various biochemical processes. For example, prostaglandin endoperoxides [1] are involved in the arachidonic cascade and antitumoral activities have been claimed for cyclic peroxyacetals isolated from marine organisms [2]. More recently, great attention has been devoted to the analysis and synthesis of arteannuin [3,4], a sesquiterpene lactone containing a peroxide linkage; this compound, common referred to as qinghaosu, extracted from *Artemisia annua L.*, is an effective antimalarial agent.

We were interested in other cyclic 1,4-epiperoxides (endoperoxides) that are the plant growth regulators designated as G factors G_1, G_2, G_3. They have been identified in leaves of *Eucalyptus grandis* and other myrtaceous plants and shown to be of general molecular structure **I** [5,6].

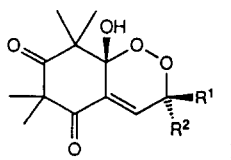
Their presence has been associated with frost resistance by controlling the active electron transport properties of membranes [7] and with stomatal conductance, photosynthesis and water loss reduction [8,9].

Based on the physiological importance of G factors, we developed a programme for the flexible chemical synthesis of the precursor syncarpic acid **II** [10] of G peroxides and their extraction from *E. grandis* [11]. We have also reported the chemical behaviour of G_3 in the presence of a Ru(II) catalyst [12], a transition metal complex that has the capability of inducing a reaction to occur via a one-electron exchange mechanism [13]. Ru(II) is a member of the iron triad and, as Fe peroxidases could be involved in the metabolism of the G peroxides, it can simulate a possible biosynthetic mechanism.

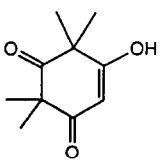
This paper reports chromatographic data for compounds obtained through the Ru(II)-catalysed reaction and a comparison between ana-

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E0804-4



I



II

	R^1	R^2
G_1	Et	Me
G_2	Me	Et
G_3	Me	Me

G_1, G_2 : 4-ethyl-1-hydroxy-4,8,8,10,10-pentamethyl-7,9-dioxo-2,3-dioxabicyclo[4.4.0]decene-5

G_3 : 1-hydroxy-4,4,8,8,10,10-hexamethyl-7,9-dioxo-2,3-dioxabicyclo[4.4.0]decene-5

lytical liquid and capillary gas chromatography (GC) in the analysis of G factors.

EXPERIMENTAL

Instrumentation

Medium-pressure liquid chromatography was performed on an Axxial apparatus (Axxial Moduloprep, Martigues, France) equipped with a refractometric detector (Jobin–Yvon R.I. Iota). Analytical liquid chromatography was performed on a Waters Model 600E apparatus equipped with a Rheodyne injector (20 μl) and a Waters 990 UV Spectroflow detector. The column used was Novapak silical gel (150 mm × 3.9 mm I.D.; particle size 4 μm) purchased from Waters. Capillary gas chromatography was performed on a Hewlett-Packard HP-5890 apparatus equipped with an HP-1 column (25 m × 0.2 mm I.D.; 0.33 μm).

Gas chromatography–mass spectrometry was performed on an HP-S 971A apparatus equipped with an HP-1 column (25 m × 0.2 mm I.D.; 0.33 μm). NMR spectra were recorded in deuterochloroform with the tetramethylsilane as reference on a Bruker AC-200 apparatus and IR spectra were recorded on a Perkin-Elmer Model 883 spectrophotometer. Melting points were measured on a Kofler bench apparatus.

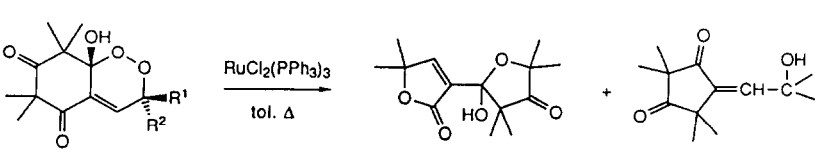
Chemicals

The three G factors (G_1, G_2, G_3) were synthesized according to the literature [11].

Ru(II)-catalysed reaction

In a round bottom two-neck flask equipped with a reflux condenser and a $CaCl_2$ tube we introduced under argon a solution of G_3 (0.375 mmol, 100 mg) in anhydrous toluene (2 ml) and $RuCl_2(PPh_3)_3$ (0.024 mmol, 100 mg) in toluene (2 ml). The mixture was stirred under reflux and the reaction was followed by TLC until complete consumption of the G_3 substrate. Thermal activation seemed necessary as no reaction occurred at room temperature. TLC on a silica gel plate with diethyl ether–light petroleum (4:6) as eluent showed the appearance of two products; one ($R_F = 0.10$) was revealed with a 10% ethanolic solution of phosphomolybdic acid and the other ($R_F = 0.22$) absorbed at a UV wavelength of 254 nm. The reaction mixture was dissolved in toluene (5 ml), passed through Celite, concentrated and purified by medium-pressure liquid chromatography [20 g of 6–35 μm silica gel (Amicon), column I.D. = 20 mm]. We obtained 68 mg (yield 68%) of compound **1** ($R_F = 0.10$) and 25 mg (yield 29%) of compound **2** (R_F = 0.22).

Compound **1**: m.p. = 142°C; IR ($CHCl_3$, ν cm^{-1}), 3500 (OH), 1750 $(C{=}O)_{lac.}$, 1740



1

Z + E forms
2a + 2b

$(C{=}O)_{ket.}$, 1600 (C=C); ^1H NMR (200 MHz, $CDCl_3$, δ ppm), 0.14, 1.28, 1.38, 1.44 (4s, 4 × 3H), 1.51 (s, 6H), 4.00 (s, 1H, OH), 7.46 (s, 1H, HC=C); ^{13}C NMR (50.32 MHz, $CDCl_3$, δ ppm), 220, 161.1, 157.7, 131.1, 103.0, 85.3, 81.3, 52.4, 26.5, 25.8, 25.1, 24.1, 27.6; MS (EI, m/z), 268 (M^+, 2%), 251 (5%), 164 (14%), 139 (10%), 112 (100%), 97 (20%), 84 (79%), 69 (78%), 43 (55%). For the X-ray structure, see ref. 11.

Compounds **2a** and **2b**: IR ($CHCl_3$, ν cm^{-1}), 3383 (OH), 1752 (C=O), 1699 (C=0), 1613 (C=C); ^{13}C NMR (50.32 MHz, $CDCl_3$, δ ppm), 219.6, 212.4, 153.2, 141.2, 123.7, 70.4, 53.4, 50.4, 53.1, 50.9, 29.5, 27.3, 21.9.

RESULTS AND DISCUSSION

The crude mixture from the Ru(II)-catalysed reaction was analysed by using capillary GC. The chromatogram obtained under optimum conditions is presented in Fig. 1a. We observe three main peaks at $t_{R1} = 2.41$ min, $t_{R2} = 4.16$ min and $t_{R3} = 8.70$ min in a ratio 29.2:8.3:62.5, respectively. After medium-pressure liquid chromatography we obtained the two fractions mentioned above corresponding to the spots observed on the TLC plate.

Capillary GC of these fractions (Fig. 1b and c) showed that the first (TLC, $R_F = 0.10$, compound **1**) corresponds to the peak at $t_{R3} = 8.70$ min and the second (TLC, $R_F = 0.22$) corresponds at two major products at $t_{R1} = 2.28$ min and $t_{R2} = 4.14$ min in a 77:23 ratio inseparable under liquid chromatographic conditions. NMR spectroscopy ($CDCl_3$ as solvent) of compounds **2a** and **2b** showed major trends in their structure (*e.g.*, ethylenic protons at $\delta = 6.31$ and 6.82 ppm and six major peaks corresponding to the methyl groups at $\delta = 1.42$, 1.29, 1.20 and 1.52, 1.47, 1.19 ppm) and their identification was performed by GC–MS. The GC–MS analysis (Fig. 2) showed that the two compounds have the same molecular mass ($m/z = 224$) and identical fragmentation patterns, in agreement with an ethylenic β-diketone structure **2**. It is noteworthy that the transformation of the cyclic G peroxides in a probably one-electron reaction leads to the major product **1**, which shows a *gem* dimethyl

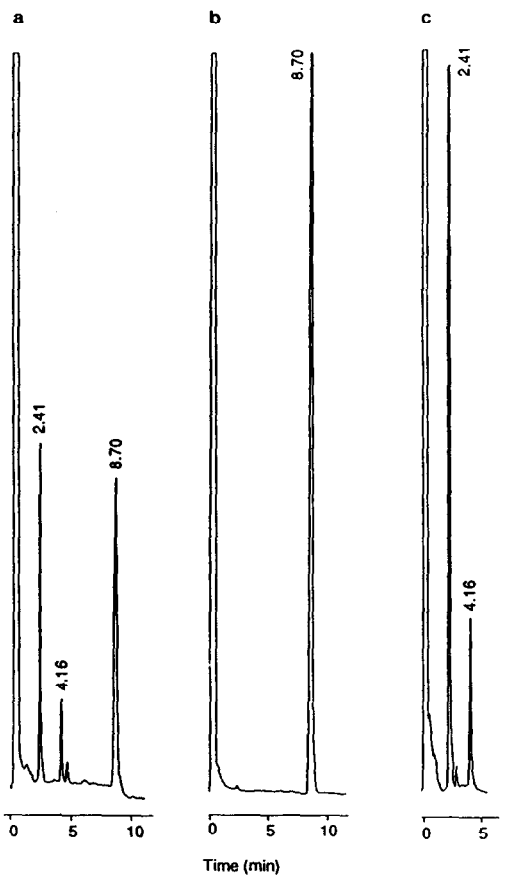


Fig. 1. Capillary GC of the Ru(II)-catalysed reaction of G_3. (a) GC of reaction mixture; (b) GC of compound **1** after purification; (c) GC of compound **2** after purification. Column, HP-1 (25 m × 0.2 mm I.D.; 0.33 μm); $T_{oven} = 150°C$; $T_{inj.} = 180°C$; $T_{det.} = 220°C$; flow-rate = 1 ml/min.

butenolide structure framework that is commonly found in various natural products extracted from plants [14–16]. It would therefore be interesting to examine further whether compounds **1** and **2** can be identified in different *Eucalyptus* species, especially those where G factors have not been identified.

As capillary GC was very efficient in the identification of the catalytic degradation products of G3, we were also interested in the comparison of the two chromatographic techniques (GC and LC) in the analysis of G_1, G_2 and G_3 peroxides. Concerning the G_1, G_2 and G_3 growth factors, the data obtained by analytical chromatography showed a clear and rapid

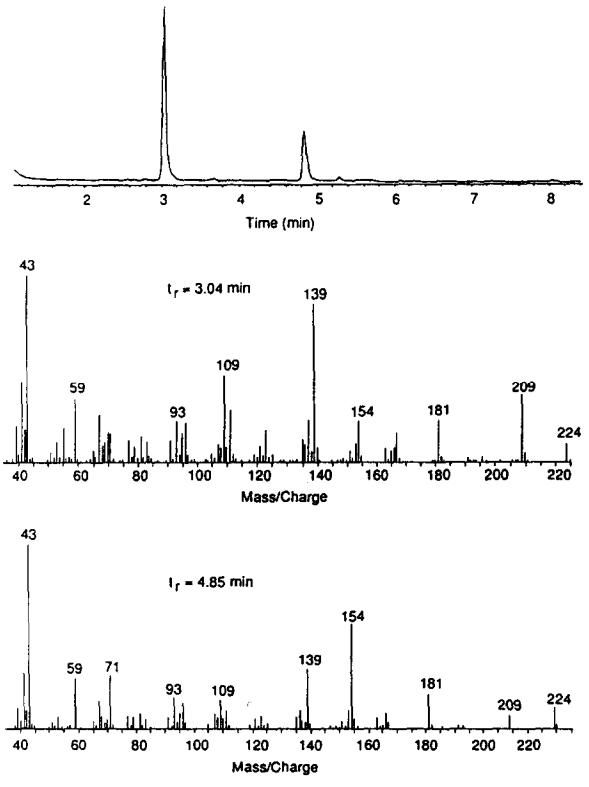


Fig. 2. GC–MS analysis of compound **2**. Column, HP-1 (25 m × 0.2 mm I.D.; 0.33 μm); $T_{oven} = 150°C$; $T_{inj.} = 180°C$; $T_{det.} = 220°C$.

differentiation of the three compounds under the experimental conditions described previously [11]. On the other hand, capillary GC of these compounds seems to be more difficult because of thermal modification of the products.

Capillary GC was performed on a BP-1 column (12 m × 0.33 mm I.D.; 0.5 μm). For G_3 ($T_{oven} = 145°C$, $T_{inj.} = 190°C$, $T_{det.} = 200°C$) a peak with retention time of 11.42 min was observed. A glass liner was added to increase the heat capacity as G_3 has a higher melting point than G_1 and G_2 (170 *versus* 100 and 127°C, respectively) [11]. Under such conditions we observed a degradation of the compound at $T_{inj.} > 190°C$.

A well defined mixture of G_1 and G_2 compounds (8:92) determined by analytical LC (Fig. 3a) was examined by capillary GC (Fig. 3b). It appears from the latter chromatogram (form of the peaks, G_1:G_2 ratio) that a good differentiation between the two compounds cannot be achieved and that G_1 and G_2 tend to equilibrate in the column. It was verified that this was due to heating; when a solid mixture of G_1 and G_2 (8:92) was heated and then measured by analytical LC, the same equilibration was observed. A correct measure of G_1 and G_2 was therefore not possible using capillary GC.

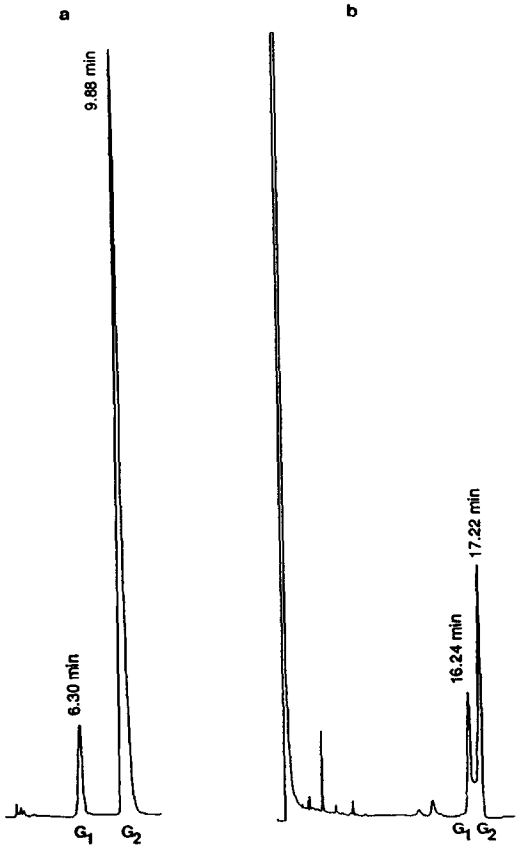


Fig. 3. Analytical HPLC and GC of an 8:92 mixture of G_1 and G_2. (a) HPLC: Novapak silica gel column (150 mm × 3.9 mm I.D.; particle size 4 μm); eluent, isooctane–methylene chloride (40:60); flow-rate, 1.5 ml/min; detection wavelength, 254 nm. (b) GC: column, BP-1 (12 m × 0.33 mm I.D.; 0.5 μm); $T_{oven} = 145°C$; $T_{inj.} = 200°C$; $T_{det.} = 200°C$; flow-rate = 1 ml/min.

CONCLUSIONS

We have described the analysis and identification by chromatographic methods of the growth factors G_1, G_2 and G_3 and the products of the Ru(II)-catalysed reaction of G_3. Whereas ana-

lytical LC can be used with success for the analysis of the G factors, capillary GC is preferred for studying and identificying the compounds obtained from the Ru(II)-catalysed reaction.

ACKNOWLEDGEMENT

Acknowledgement is made to the Centre National de la Recherche Scientifique for support of this work.

REFERENCES

1 B. Samuelson, *Angew. Chem., Int. Ed. Engl.*, 22 (1983) 805.
2 B.B. Snider and Z. Shi, *J. Org. Chem.*, 55 (1990) 5669.
3 G. Schmid and W. Hofheinz, *J. Am. Chem. Soc.*, 105 (1983) 624.
4 M.A. Avery, C. Jennings-White and W.K.M. Chong, *Tetrahedron Lett.*, 28 (1987) 4629.
5 W.D. Crow, W. Nicholls and M. Sterns, *Tetrahedron Lett.* (1971) 1353.
6 M. Sterns, *J. Cryst. Mol. Struct.*, 1 (1971) 373.
7 D.M. Paton, *Aust. J. Bot.*, 29 (1981) 675.
8 T.D. Sharkey, G.F. Stevenson and D.M. Paton, *Plant Physiol.*, 69 (1982) 935.
9 D.M. Paton, A.K. Dhawan and R.R. Willing, *Plant Physiol.*, 66 (1980) 254.
10 M. Benbakkar, M. Baltas, L. Gorrichon and J.P. Gorrichon, *Synth. Commun.*, 19 (1989) 3241.
11 M. Baltas, M. Benbakkar, L. Gorrichon and C. Zedde, *J. Chromatogr.*, 600 (1992) 323.
12 M. Baltas, M. Benkakkar and L. Gorrichon, *J. Chem. Soc., Chem. Commun.*, (1991) 1044.
13 M. Suzuki, H. Ohtake, Y. Kameya, N. Hamanaka and R. Noyori, *J. Org. Chem.*, 54 (1989) 5292.
14 F.R. Kinder and A. Padwa, *Tetrahedron Lett.*, 31 (1990) 6835.
15 R. Bloch and L. Gilbert, *J. Org. Chem.*, 52 (1987) 4603.
16 R.M. Cory, B.M. Ritchie and A.M. Shrier, *Tetrahedron Lett.*, 31 (1990) 6789.