139. A Novel Allenic Epoxycyclohexane and Related Compounds from *Eutypa lata* (Pers: F.) TUL.

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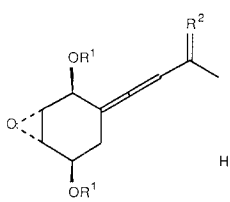
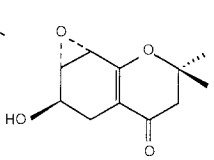
The novel allenic epoxycyclohexanes **1**, and related compounds **2** and **3** have been isolated from the culture medium of *Eutypa lata*. Their structures were established by a combination of spectroscopic and chemical techniques. Biogenetic origin and biological activity of these compounds are also discussed.

Introduction. – *Eutypa lata* is one of the pathogenic agents responsible for the vineyard die-back observed during the last few years in Switzerland and France [1–3]. This fungus attacks many other woody species and appears to be widely distributed in several other countries [4–6]. Biological studies have shown that the disease is linked to a toxic secondary fungal metabolite, which is transfered by the sap to the new branches, causing withering, leading to death [7]; it is known as 'dying arm' disease or 'eutypiosis', and at present there are no means of controlling it. In the course of our search for secondary metabolites with a phytotoxic activity in the culture medium of *Eutypa lata,* we reported the isolation of several new aromatic compounds with a 3-methylbut-3-en-1-ynyl substituent [8]. Other compounds were also isolated. The most abundant was the novel allenic derivative **1** which is probably biogenetically related to the acetylenic compounds and is presumably a precursor of the tetrahydrochromanone derivatives **2** and **3**.



1 $R^1 = H$, $R^2 = CH^2$
1a $R^1 = Ac$, $R^2 = CH_2$
1b $R^1 = Ac$, $R^2 = O$



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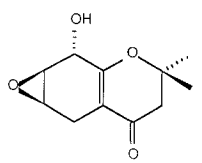

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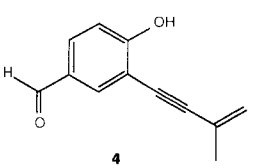

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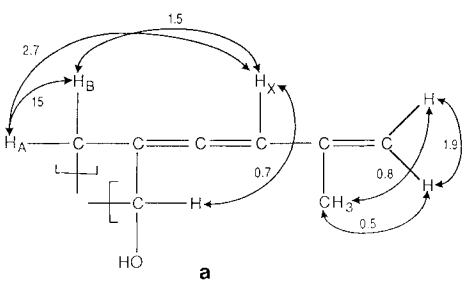
Here, we report the structure elucidation, biological activity and suggest a biosynthetic relationship between these new compounds.

Results and Discussion. – *Eutypa lata* was grown *in vitro* for six weeks. The mycelium was removed by filtration and the culture medium extracted with Et_2O after adjustment of pH to 4.5. The Et_2O extract was purified by chromatography on silica gel using $CHCl_3$/MeOH as eluent. Several fractions of increasing polarity were collected. A series

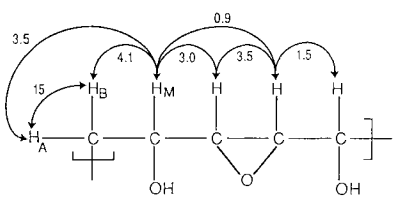
of aromatic acetylenic metabolites was isolated [8]; among these, compound **4** showed the highest phytotoxic activity in our bioassay. Allenic compound **1** and the tetrahydrochromanone derivatives **2** and **3** were isolated from the more polar fractions, after further purification by semi-preparative HPLC on a *RP-18* column.

Molecular composition of **1** was established as $C_{11}H_{14}O_3$ by high-resolution mass spectrometry. The IR spectrum showed the very characteristic allenic absorption band at 1950 cm^{-1}. A signal for an sp-C-atom at 205.35 ppm in the ^{13}C-NMR spectrum supported this hypothesis [9]; the other sp^2-C-atoms of the allenic group appeared at 101.49 *(s)* and 99.36 *(d)* ppm. In this spectrum, signals of a Me group (19.95 ppm; *q*), of a sp^3-CH_2 group (31.82 ppm; *t*), of two methine sp^3-C-atoms of an oxyrane ring (57.15 *(d)*, 57.52 *(d)* ppm), of two methine sp^3-C-atoms bearing OH group (66.92 *(d)*, 68.38 *(d)* ppm), and of two sp^2-C-atoms of a vinyl terminal group (114.15 *(t)*, 140.50 *(s)* ppm) were also present. In the ^{1}H-NMR spectrum, a broad peak, corresponding to two exchangeable protons, appeared at 2.15 ppm. Acetylation of **1** gave a diacetyl derivative **1a**. In the ^{1}H-NMR spectrum of **1a**, the two signals at 4.56 and 4.32 ppm in **1** were shifted downfield to 5.62 and 5.18 ppm respectively. Decoupling experiments on the Me signal at 1.75 ppm revealed a coupling with the olefinic signals at 4.99 and 4.92 ppm.

Oxidation of the double bond in **1a** with $OsO_4/NaIO_4$ gave compound **1b**, in the ^{1}H-NMR spectrum of which the olefinic-proton signals had disappeared, and the Me signal had shifted downfield to 2.23 ppm establishing the presence of a terminal methylvinyl group. The signal at 6.18 ppm was assigned to the allenic proton; this exhibited allylic couplings with the signals of the *ABMX* spin system of CH_2 protons centred at 2.32 ppm and 2.49 ppm ($J_{AX} = 2.7$, $J_{BX} = 1.5$ Hz) and *m* at 4.56 ppm ($J = 0.7$ Hz).



a



b

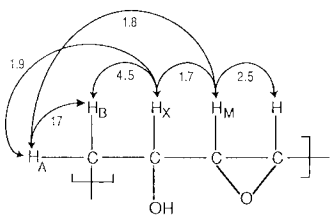
Irradiation of the signal at 4.32 ppm revealed couplings with the epoxide proton at 3.25 ppm and the *ABMX* spin system ($J_{AM} = 3.5$, $J_{BM} = 4.1$ Hz). The structural fragments **a** and **b** were, thus, established, and the cyclic structure of 5-(3-methylbuta-1,3-dienylidene)-2,3-epoxycyclohexane-1,4-diol (**1**) was proposed. The small values observed led us to propose a *trans* pseudoaxial arrangement for the OH and oxirane moieties in the substituted cyclohexane skeleton. This structure was confirmed by X-ray analysis.

From the X-ray analysis of **1**, it can be seen that the six-membered ring is best described in terms of a sofa conformation; atom C(6) being displaced by 0.627(3) Å from the best least-squares plane through the remaining five atoms (planar to within 0.053(1) Å). The best least-squares plane containing atoms C(1), C(2), C(6), C(7), and C(8)

(planar to within 0.006(3) Å) is inclined to the best least-squares plane containing atoms C(7), C(8), C(9), C(10), and C(11) (planar to within 0.033(3) Å) by an angle of 86.6°. The average allene bond length is 1.311(3) Å, and the angle C=C=C is 178.5(3)°. The geometry of the oxirane ring is as expected, the average ring angle being 60.0(1)°. Molecules related by the 2-fold screw axis, parallel to axis *b*, are linked by an intermolecular H-bond between OH proton H(01) and the OH O-atom O(3) of the symmetry-related molecule.

Molecular composition of **2** was established as $C_{11}H_{14}O_4$. The IR spectrum contained an absorption band at 1665 cm^{-1}, characteristic of a cyclic α,β-unsaturated ketone. In the ^{1}H-NMR spectrum, signals for two non-coupled Me groups appeared at 1.42 and 1.49 ppm; one *AB* and an *ABMX* spin system were also present. The *AB* system was centred at 2.50 and 2.67 ppm (J_{AB} = 16 Hz). The other was more complicated due to two secondary couplings; the high-field part of this system centred at 2.22 ppm was a *dd* (J_{AB} = 17, J_{BX} = 4.5, J_{BM} = 0 Hz); the second part centred at 2.85 ppm was 2 *dd* (J_{AB} = 17, J_{AX} = 1.90, J_{AM} = 1.80 Hz). The high J_{AB} values in these systems indicated two protons with geminal coupling.

Acetylation of **2** gave a monoacetyl derivative; the signal at 4.56 ppm was shifted downfield to 5.60 ppm. Decoupling experiments revealed that the signal at 4.56 ppm was coupled with the signal at 3.65 ppm (epoxy group), and the *ABMX* spin system (J_{AX} = 1.9, J_{BX} = 4.5 Hz). Irradiation of the signal at 3.65 ppm revealed couplings with the signals at 4.56 ppm and 3.35 ppm, and a long-range W-type coupling with one signal of the *ABMX* system (J_{AM} = 1.80 Hz). The structural fragment **c** was, thus, established, and the structure of 6-hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-7,8-epoxychroman-4-one (**2**) is proposed.



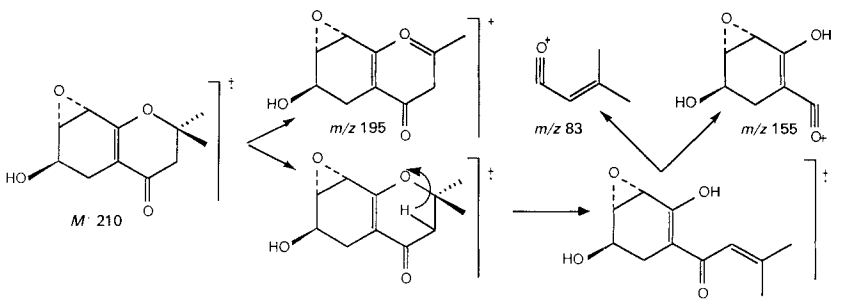
c

The relative configuration shown in **2** was deduced from ^{1}H-NMR coupling constants with the aid of *Dreiding* models [10]. The coupling constants (J = 1.70, J = 1.90, and J = 4.50 Hz) shown in **c**, between the signal corresponding to the proton geminal to the OH group and the signals of the protons in the oxirane ring, and the *ABMX* spin system due to the CH_2 group, are characteristic of two pseudo-equatorial-equatorial and a pseudo-equatorial-axial couplings, respectively. Therefore, in the 'half-chair' conformation of the 7-oxabicyclo[4.1.0]hept-3-ene ring system, the OH and epoxy group must be in a pseudo-axial positions. In this conformation, the coupling between the bridgehead proton and the *ABMX* system is explained by a W-type long-range coupling. Compound **3** appeared to be closely related to **2**, since their IR and mass spectra were very similar. The ^{1}H-NMR was compared to that of **2**. Modifications in the oxygenated ring of the molecule were observed; the signal of the proton geminal to the OH group was shifted

upfield to 4.50 ppm and showed a long-range coupling with the *ABMX* spin system (J_{BX} = 1.8 Hz, homoallylic coupling) which appeared as 2 *dd*, one at 2.60 ppm (J_{AB} = 16, J_{BM} = 1.5, J_{BX} = 1.8 Hz) and the other at 3.05 ppm (J_{AB} = 16, J_{AM} = 1.5, J_{AX} = 0.7 Hz). Irradiation of the *ABMX* system revealed small coupling between it and the highfield part of the bridgehead proton system (J_{AM} = 1.5, J_{BM} = 1.5 Hz) which appeared as 2 overlapping *m* at 3.45 and 3.40 ppm. We propose the structure of 8-hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-6,7-epoxychroman-4-one (**3**) for this compound, which can be viewed as an isomer of **2**.

The proposed structures **2** and **3** are in good agreement with their mass spectra. The M^+ undergoes Me group elimination; $[M - CH_3]^+$ ion represented the base ion in the mass spectrum. Other characteristic fragments were at *m/z* 155 and *m/z* 83, for which we propose the fragmentation pathway indicated in *Scheme 1*.

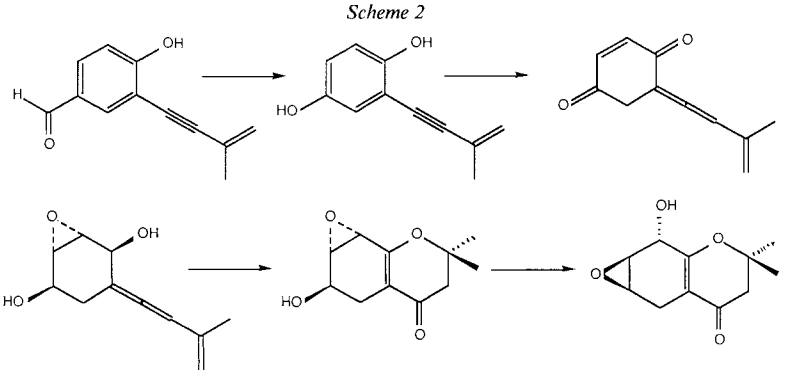
Scheme 1



We verified this proposal by comparison of the collisional activated spectra of ion at *m/z* 83 with that obtained for the same ion present in the mass spectrum of 3,3-dimethylpropenoic acid.

The three new compounds described here can be reasonably related to the acetylenic compounds isolated from *Eutypa lata* [8] by the biogenetic pathway illustrated in *Scheme 2*. Compound **1** can, therefore, be considered as the key intermediate in the transformation of the acetylenic compounds into tetrahydrochromanone derivatives.

Scheme 2



Compounds **1**, **2**, and **3**, showed no phytotoxic activity in our bioassays. Compound **1** has a large structural similarity with the recently described asperpentyne [11]. Highly oxygenated cyclohexanoid compounds have been reported to show a wide range of biological activities [12]. However, in our case **1** showed low antimitotic (tubuline test) and antibacterial activity. Synthesis of these compounds and biosynthetic studies with labelled precursors are in progress.

We are grateful to Prof. *G. Ourisson* (Gif-sur-Yvette) for biological testing, to Dr. *P. Pezet* (Swiss Agricultural Research Station, Changin) for the culture of the fungus and bioassays, and to Miss *C. Lachat* for typewriting this manuscript. Financial support by the *Swiss National Science Foundation* (project No. 2.043–0.86) is gratefully acknowledged.

Experimental Part

General. See [8].

5-(3-Methylbuta-1,3-dienylidene)-2,3-epoxycyclohexane-1,4-diol (**1**): 8 mg of amorphous white solid. R_f (silica gel, $CHCl_3$/MeOH 9:1) 0.50. UV (MeOH): λ_{max} 225. IR: 3400 (OH), 2920 (=CH_2), 1950 (allenic group), 1620 (C=C), 1420, 1250 (epoxy group), 1080 (C=C), 1010 (OH), 890 (=CH_2). ^{1}H-NMR: 1.75 (*dd*, 3H); 2.32 (*dd*, 1H); 2.49 (*ddd*, 1H); 3.25 (*m*, 2H); 4.32 (*m*, 1H); 4.56 (br.*s*, 1H); 4.92 (br.*s*, 1H); 4.99 (br.*s*, 1H); 6.18 (br.*s*, 1H). ^{13}C-NNR: 19.95 (*q*); 31.82 (*t*); 57.15 (*d*); 57.52 (*d*); 66.92 (*d*); 68.38 (*d*); 99.36 (*d*); 101.49 (*s*); 114.15 (*t*); 140.50 (*s*); 205.35 (*s*). EI-MS: 194 (43, M^+), 179 (7), 161 (10), 147 (25), 133 (22), 122 (35), 121 (32), 107 (23), 105 (20), 93 (33), 91 (100), 83 (44), 79 (50), 77 (55), 73 (25), 65 (22), 41 (42).

X-Ray Analysis. Suitable crystals were grown from $CHCl_3$ · *Crystal Data.* Space group $P2_12_12_1$, $a = 6.225\,(8)$, $b = 7.145\,(8)$, $c = 23.38\,(1)$ Å, $V = 1039.9$ Å^3, $Z = 4$, $D_x = 1.239$ mg·m^{-1}, MoK_α, $\lambda = 0.7107$ Å, $\mu = 0.53$ cm^{-1}. Two crystals of similar dimensions (0.15 × 0.25 × 0.40 mm) were used for data collection. Preliminary *Weissenberg* and precession photographs indicated the crystals to be of the orthorhombic space group $P2_12_12_1$. Intensity data for layers $h = 0$–4 and $k = 0$–5 and with $\theta \leq 25°$ were measured on a *Stoe STADI-2* two-circle diffractometer

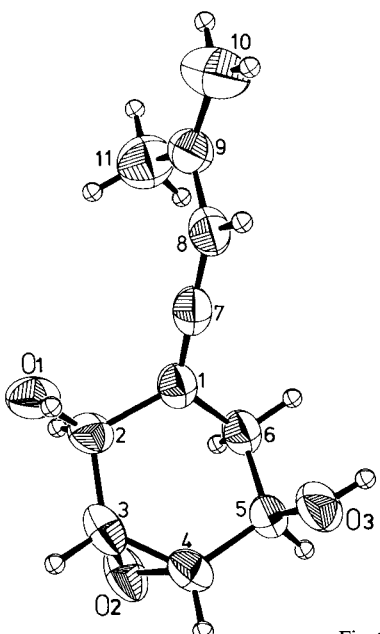


Fig. *ORTEP Plot of* **1**

(graphite-monochromated MoK_α radiation). A total of 1549 reflections were measured using the variable ω-scan technique [13]; of the 903 unique reflections ($R_{INT} = 0.011$), 878 were considered observed ($F_o > 4\sigma F_o$). The structure was solved by direct methods using the SHELX-76 program [14], which was used for all further calculations. In the final cycles of least-squares refinement, H-atoms located from difference maps were included and refined isotropically. Weighted anisotropic full-matrix least-squares refinement for 878 reflections converged at $R = 0.033$, $R_w = 0.035$, where $w = [\sigma^2(F_o) + 0.00062(F_o^2)]^{-1}$. Average param. shift/e.s.d. < 0.05. Heights in final difference map +0.11 to −0.13 e·Å^{-3}. Final position and thermal parameters, bond distances, and bond angles have been deposited with the Cambridge Crystallographic Data Centre, Lensefield Road, Cambridge CB2 1EW, U.K. The ORTEP [15] plot is given in the *Figure*.

5-(3-Methylbuta-1,3-dienylidene)-2,3-epoxycyclohexane-1,4-diyl Diacetate (**1a**). To a soln. of **1** (1 mg) in anh. pyridin (1 ml), Ac_2O (2 ml) was added. The reaction was quenched by MeOH added after 12 h. Pyridine was removed under vacuum, and the final product was purified by HPLC on *RP-18* column. ^{1}H-NMR: 1.75 (*dd*, 3H); 2.12 (*s*, 6H); 2.30 (*dd*, 1H); 2.51 (*ddd*, 1H); 3.27 (*m*, 2H); 4.88 (br.*s*, 1H); 4.98 (br.*s*, 1H); 5.18 (*m*, 1H); 5.62 (br.*s*, 1H); 6.10 (*m*, 1H). EI-MS: 278 (8, M^+), 218 (12), 176 (63), 161 (12), 107 (18), 79 (36), 77 (25), 43 (100).

5-(3-Oxobut-1-enyliden)-2,3-epoxycyclohexane-1,4-diyl Diacetate (**1b**). To a soln. of **1a** (1 mg) in Et_2O (1 ml), OsO_4 (1 ml of a 1% Et_2O soln.) and $NaIO_4$ (1 ml of a 10% AcOH soln.) were added. The reaction was monitored by TLC. The mixture was diluted with H_2O, extractd with Et_2O, and the final product was purified by HPLC on *RP-18* column. ^{1}H-NMR: 2.12 (*s*, 6H); 2.23 (*s*, 3H); 2.42 (*dd*, 1H); 2.65 (*ddd*, 1H); 3.37 (*m*, 2H); 5.36 (*m*, 1H); 5.78 (br.*s*, 1H); 5.82 (*m*, 1H). EI-MS: 280 (5, M^+), 238 (4), 220 (6), 196 (7), 195 (8), 178 (70), 162 (40), 161 (30), 152 (72), 135 (47), 118 (30), 107 (48), 79 (20), 77 (18), 43 (100).

6-Hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-7,8-epoxychroman-4-one (**2**): 2 mg of amorphous white solide. R_f (silica gel, $CHCl_3$/MeOH 9:1) 0.6. UV (MeOH): λ_{max} 285. IR: 3400 (OH), 2900 (CH_3), 1665 (C=O), 1610 (C=C), 1450, 1250 (epoxy ring), 1160, 910, 790. ^{1}H-NMR: 1.42 (*s*, 3H); 1.49 (*s*, 3H); 2.22 (*dd*, *B* of *ABMX*, $J_{AB} = 17$, $J_{BX} = 4.50$, $J_{BM} = 0$, 1H); 2.50, 2.67 (*AB*, $J_{AB} = 16$, 2H); 2.85 (*ddd*, *A* of *ABMX*, $J_{AB} = 17$, $J_{AX} = 1.90$, $J_{AM} = 1.80$, 1H); 3.35 (*d*, $J = 2.5$, 1H); 3.65 (*m*, 1H); 4.56 (*m*, 1H). EI-MS: 210 (26, M^+), 195 (100), 155 (32), 123 (12), 83 (22), 71 (20), 56 (18).

8-Hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-6,7-epoxychroman-4-one (**3**): 1 mg of amorphous white solid. R_f (silica gel, $CHCl_3$/MeOH 9:1) 0.6. UV (MeOH): λ_{max} 285. IR: 3400 (OH), 2900 (CH_3), 1660 (C=O), 1610 (C=C), 1450, 1250 (epoxy ring), 1150, 910, 780. ^{1}H-NMR: 1.38 (*s*, 3H); 1.45 (*s*, 3H); 2.45, 2.63 (*AB*, $J_{AB} = 16$); 2.60 (*ddd*, *B* of *ABMX*, $J_{AB} = 17$, $J_{BM} = 1.50$, $J_{BX} = 1.80$, 1H); 3.05 (*ddd*, *A* part of *ABMX*, $J_{AB} = 17$, $J_{AM} = 1.50$, $J_{AY} = 0.70$); 3.40 (*m*, 1H); 3.45 (*m*, 1H); 4.50 (*m*, 1H). EI-MS: 210 (34, M^+), 195 (100), 177 (6), 155 (28), 123 (8), 83 (20), 71 (18), 56 (25).

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