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neo-Clerodane diterpenoids from *Baccharis flabellata*

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Abstract

Three diterpenoid derivatives were isolated from the acetone extract of *Baccharis flabellata*. Their structures were elucidated as 2,19;15,16-diepoxy-*neo*-clerodan-3,13(16),14-trien-18-oic acid, 15,16-epoxy-5,10-*seco*-clerodan-1(10),2,4,13(16),14-pentaen-18,19-olide and 15,16-epoxy-*neo*-clerodan-1,3,13(16),14-tetraen-18,19-olide through spectroscopic analyses.

Keywords: *Baccharis flabellata*; Asteraceae; *neo*-Clerodane-type; Diterpenoids

1. Introduction

In a previous communication, we reported the isolation of two *neo*-clerodane diterpenoids, flabeloic acid (**1**) and a 5,10-*seco*-clerodane diterpenoid derivative (**2**) from the aerial parts of *Baccharis flabellata* Hook. & Arn. var. *flabellata* (Asteraceae) (Saad et al., 1988). As part of our search for new clerodane diterpenoids, we reinvestigated the acetone extract of this species. The isolation and structural elucidation of three new *neo*-clerodane diterpenoids is reported in this contribution.

2. Results and discussion

Repeated chromatography of an acetone extract of the aerial parts of *B. flabellata* var. *flabellata* (see Experimental) provided the previously isolated compounds **1** and **2**, together with three new clerodanes **3, 4** and **5**. The HREIMS of **3** displayed a $[M^+]$ at m/z 330.181297 corresponding to the molecular formula $C_{20}H_{26}O_4$ (calc. 330.193110). However, compound **3** was purified as its methyl ester derivative **3a**, this being obtained as a colourless dextrorotatory oil, whose molecular formula, $C_{21}H_{28}O_4$, was deduced by mass spectral and NMR spectral data analyses (Tables 1, 2 and 3). The ^{13}C NMR spectrum (Table 2) and DEPT experiments revealed the presence of 21 non-equivalent carbons. Among the 14 sp^3 hybridized carbons present in the extract, three correspond to methyl groups, six to methylene groups, three to methine groups, and two are quaternary carbons. Additionally, the six sp^2 hybridized carbons are represented by four methine groups and two quaternary carbons. The signal of a carbonylic carbon comes from the ester moiety.

The six vinylic (or sp^2) carbons were consistent with the presence of three double bonds in the molecule. The molecular formula, $C_{21}H_{28}O_4$, of compound **3a** defined a degree of unsaturation of eight, which suggested that it contained four rings in addition to the three double bonds and one carbonyl group. The above data agreed with a clerodane skeleton. Its IR spectrum showed a furyl group (1506 and 873 cm^{-1}). Signals of four carbons at δ 125.3 (*s*, C-13), 110.9 (*d*, C-14), 142.7 (*d*, C-15) and 138.3 (*d*, C-16) in the ^{13}C NMR spectrum, together with the corresponding signals of three aromatic protons in the ^1H NMR (Table 1), indicated the presence of a β-substituted furan ring. The mass spectrum of **3** (see Experimental) exhibited a small $[M^+]$ peak at m/z 330. The major peak at m/z 218 was obtained according to Scheme 1. The presence of a β-ethylfuran side chain showed the occurrence of peaks at m/z 95 and 81,

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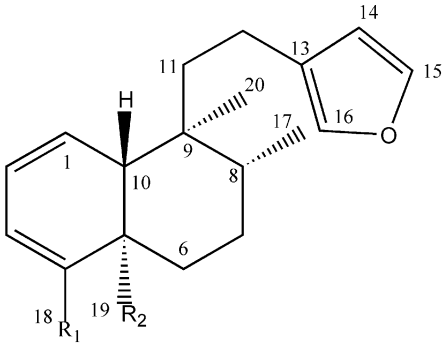
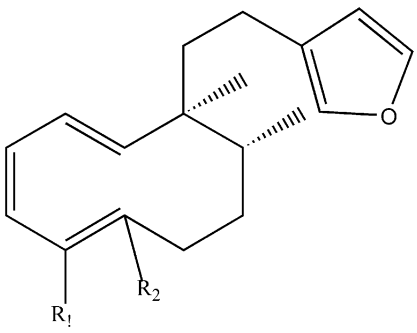


1 R_1 = COOH; R_2 = CH_2OAc

5 R_1-R_2 = (C=O)-O-CH_2



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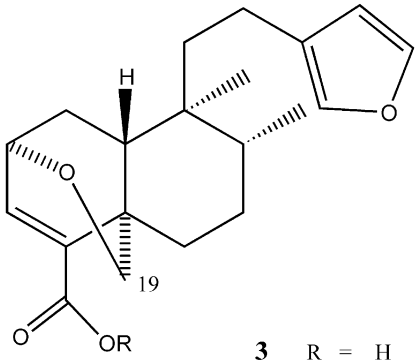


3 R = H

3a R = Me

Table 1
^{1}H NMR spectral data of compounds **3a**, **4** and **5** ($CDCl_3$, TMS, δ values in ppm, J values in Hz)

H	3a	4	5
1	1.98 (*m*)	5.86 (*d, J* = 16.8)	6.23 (*ddd, J* = 9.6, 2.2, 1.0)
2	4.50 (*ddd, J* = 5.6, 3.7, 1.6)	6.30 (*dd, J* = 11.7, 2.9)	6.17 (*ddd, J* = 9.6, 4.8, 3.1)
3	7.10 (*d, J* = 5.6)	6.02 (*brd, J* = 11.7)	6.93 (*brd, J* = 4.8)
6		3.11 (*brs*)[a]	
		2.21 (*m*)[b]	
10		5.77 (*dd, J* = 16.8, 2.9)	2.44 (*dd, J* = 3.1, 2.2)
14	6.18 (*brs*)	6.23 (*brs*)	6.50 (*brs*)
15	7.31 (*m*)	7.32 (*m*)	7.42 (*m*)
16	7.15 (*m*)	7.17 (*m*)	7.27 (*m*)
17	0.88 (*d, J* = 6.5)	1.04 (*d, J* = 7.0)	1.06 (*d, J* = 7.4)
19	2.95 (*dd, J* = 8.4, 1.6)	4.73 (*d, J* = 17.5)	4.61 (*d, J* = 8.3)
	4.14 (*d, J* = 8.4)	4.62 (*d, J* = 17.5)	4.03 (*dd, J* = 8.3, 1.9)
20	0.99 (*s*)	0.87 (*s*)	1.15 (*s*)
COOMe	3.74 (*s*)		

[a] Overlapped with H-12.
[b] Signal affected by exchange phenomena.

Table 2
^{13}C NMR (DEPT) spectral data of compounds **3a**, **4** and **5** ($CDCl_3$)

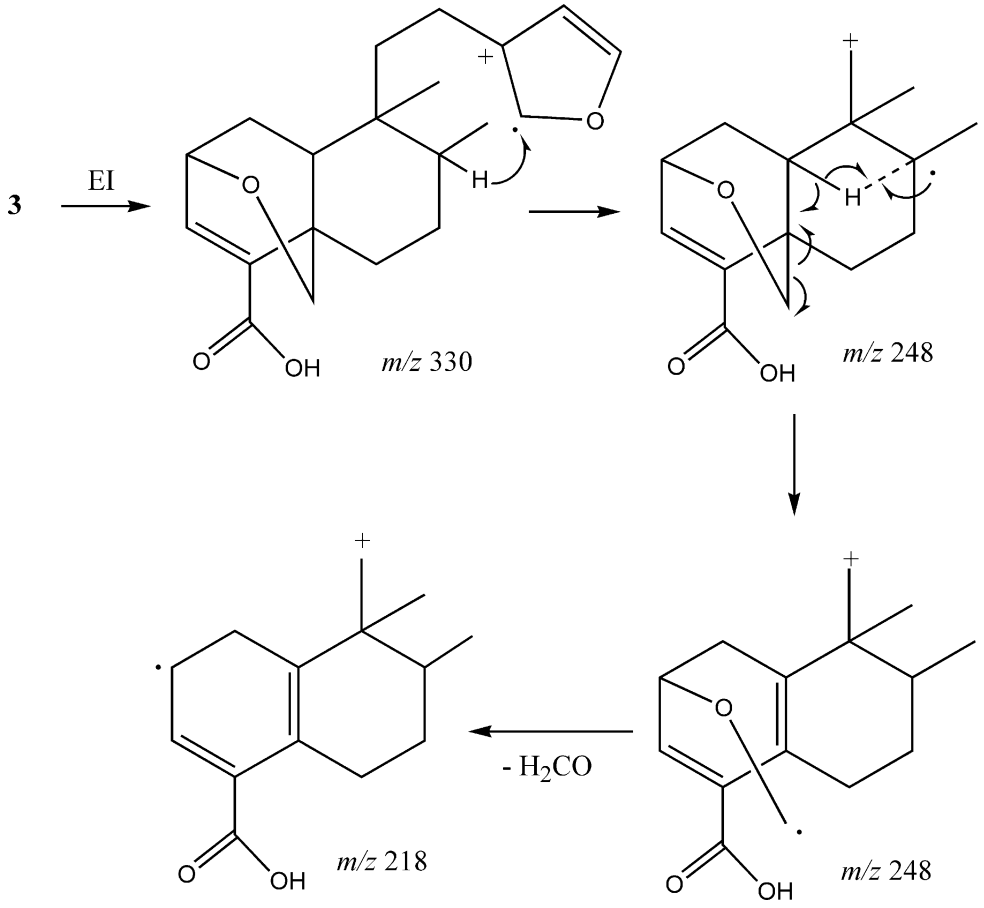
C	3a	4	5
1	28.2 (*t*)	123.9 (*d*)[a]	136.6 (*d*)
2	66.2 (*d*)	133.2 (*d*)	121.8 (*d*)
3	139.3 (*d*)	119.7 (*d*)	128.6 (*d*)
4	143.1 (*s*)	123.3 (*s*)[a]	131.5 (*s*)
5	39.6 (*s*)	158.4 (*s*)[a]	39.8 (*s*)
6	18.3 (*t*)	28.7 (*t*)	22.3 (*t*)
7	27.0 (*t*)	33.2 (*t*)[a]	23.8 (*t*)
8	36.4 (*d*)	35.2 (*d*)[a]	35.5 (*d*)
9	39.3 (*s*)	41.9 (*s*)	37.5 (*s*)
10	38.3 (*d*)	144.7 (*d*)	46.1 (*d*)
11	39.7 (*t*)	40.7 (*t*)	41.9 (*t*)
12	28.9 (*t*)	19.8 (*t*)	19.7 (*t*)
13	125.3 (*s*)	125.3 (*s*)	124.6 (*s*)
14	110.9 (*d*)	110.9 (*d*)	110.6 (*d*)
15	142.7 (*d*)	142.7 (*d*)	143.0 (*d*)
16	138.3 (*d*)	138.4 (*d*)	138.3 (*d*)
17	15.7 (*q*)	17.3 (*q*)	16.2 (*q*)
18	165.3 (*s*)	174.9 (*s*)[a]	169.3 (*s*)
19	68.0 (*t*)	71.6 (*t*)	77.4 (*t*)
20	16.5 (*q*)	13.9 (*q*)[a]	22.2 (*q*)
OMe	51.5 (*q*)		

[a] Signals affected by exchange phenomena.

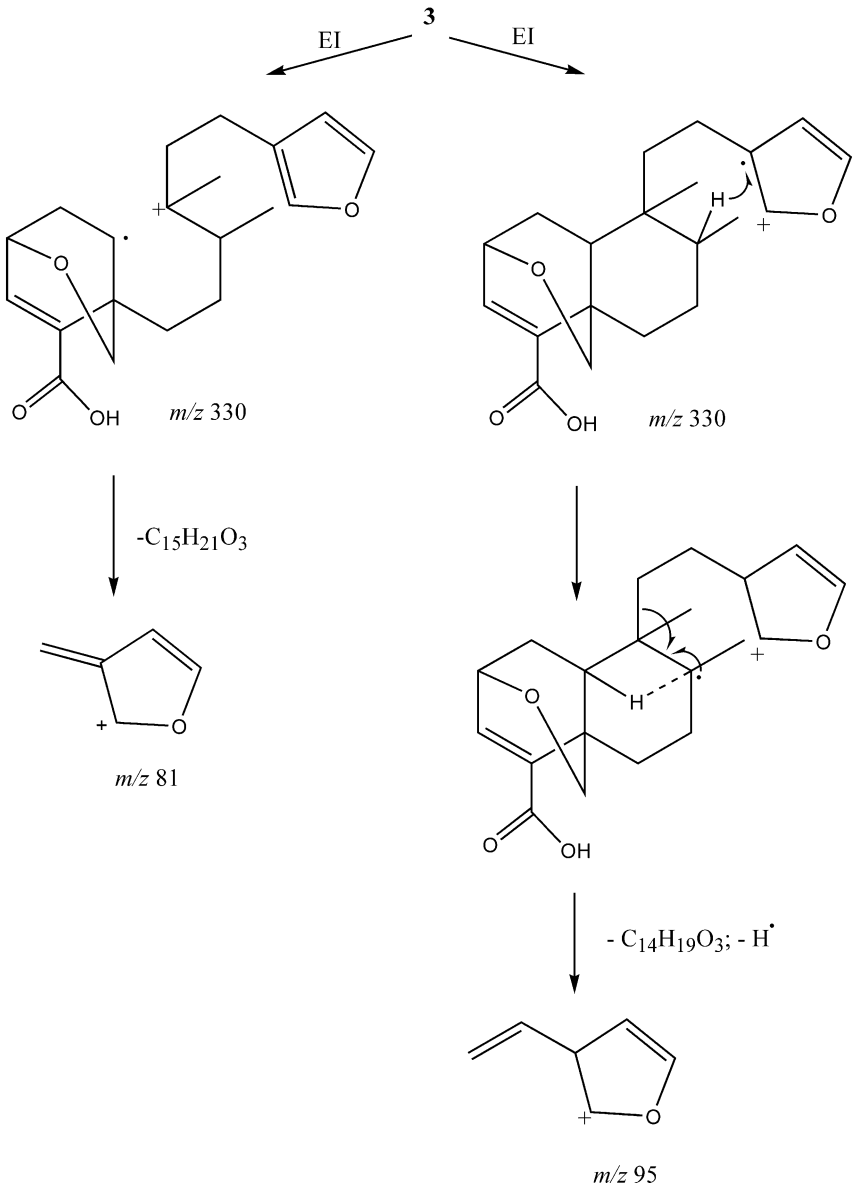
according to Scheme 2 (Bruno et al., 1999). On the other hand, a quaternary carbon at 165.3 (*s*, C-18) and a methoxyl group at 51.5 (*q*, OMe) clearly connected in **3a** (Table 3), suggested the presence of a carboxymethyl group. The UV spectrum absorption maximum at 207 nm with a shoulder at 221 nm, and IR absorption at ν_{max} 1716 cm^{-1} suggested the presence of an α,β-unsaturated carboxymethyl group. The signal at δ 7.10 (*d*)

Table 3
Selected COLOC correlations for compounds **3a** and **4**

3a		4	
C	Long-range correlations	C	Long-range correlations
2	H-3	1	H-10
3	H-1	2	H-1
4	H-2, H-19	3	H-1
7	H-17	4	H-2, H-19
8	H-17, H-20	7	H-17
9	H-17, H-20	9	H-10, H-17, H-20
10	H-2	10	H-1
18	H-3, OMe	18	H-19



Scheme 1.



Scheme 2.

was assigned to the vinylic proton H-3 which belongs to the aforementioned α,β-unsaturated system (Zdero et al., 1992). This proton showed vicinal coupling with a H-2β proton in the ^{1}H–^{1}H COSY spectrum and long-range coupling with C-18 and C-2 (δ 66.2) from the 2D COLOC experiments (Table 3). Thus, the oxygen-bearing methine carbon must be adjacent to a vinylic proton at C-3. In addition, two signals of the oxymethylene group at δ 4.14 (*d*, $J=8.4$ Hz) and 2.95 (*dd*, $J=8.4$, 1.6 Hz), were assigned to H-19 *pro*-R and H-19 *pro*-S respectively, in which H-19 *pro*-S showed the J long-range W-coupling ($J=1.6$ Hz) with H-10 proton. The above mentioned spectroscopical data showed that an ether bridge was established between C-2 and C-19 (Cuevas et al., 1987).

The relative stereochemistry was confirmed by analysis of the NOESY spectrum, where a cross peak between H-20/H-19 *pro*-S was observed. These results can be rationalized only if C-20 and C-19 are on the same face of the molecule. Therefore, the structure of **3** was elucidated to be 2,19;15,16-diepoxy-*neo*-clerodan-3,13(16),14-trien-18-oic acid.

The absolute stereochemistry of **3** was not ascertained. However, taking into account biogenetic considerations, it is reasonable to assume it belongs to the *neo*-clerodane series like other diterpenoids isolated from *Baccharis* species (De La Torre et al., 1997).

Compound **4** was isolated as a colourless levorotatory oil. Its molecular formula was deduced as $C_{20}H_{24}O_3$ from its HREI MS (see Experimental) and NMR spectroscopic data. The structural elucidation of **4** was initially hampered by conflicting ^{13}C NMR spectral data which showed broadened and flattened signals due to

exchange NMR phenomena (Sandström, 1982). The ^{1}H NMR spectrum of **4** (Table 1) revealed a typical resonance pattern due to the presence of a β-substituted furan ring, together with the corresponding signals of four aromatic carbons in the DEPT spectra. Four proton signals clearly coupled at δ 6.30 (*dd*, H-2), 6.02 (*brd*, H-3), 5.86 (*d*, H-1) and 5.77 (*dd*, H-10) must be attributed to the occurrence of a conjugated triene system, which can only be accommodated in a 5,10-*seco*-clerodane skeleton (Saad et al., 1988). COLOC cross peaks (Table 3) of the methyl group at δ 0.87 (*s*, Me-20) with C-10 (δ 144.7) confirmed that assertion. The NMR spectral data and the molecular formula suggested that **4** was an analogue of **2,** in which the acetoxymethylene group on C-5 was replaced by a oxymethylene group of a γ-lactone, although the H-19 AB system was shifted to δ 4.73 and 4.62 (both *d*, $J = 17.5$ Hz). Moreover, a long range coupling with H-3, H-2 and H-1 was evident from the ^{1}H–^{1}H COSY spectrum, and correlations with C-18 (δ 174.9) and C-4 (δ 123.3) were demonstrated by COLOC spectrum. On the other hand, the IR (1753, 1236, 1159 cm^{-1}) and UV (280 nm shoulder) absorptions suggested a conjugated system and a lactone ring. On the basis of these findings and other COLOC correlations, we assigned **4** to be 15,16-epoxy-5,10-*seco*-clerodan-1(10),2,4,13(16),14-pentaen-18,19-olide.

Compound **5** has a molecular formula of $C_{20}H_{24}O_3$, as evidenced from its HREIMS (see Experimental) and NMR spectral data (Tables 1, 2 and 3). Of the 20 carbons appearing in the ^{13}C NMR spectrum, two belong to methyl, five to methylene and eight to methine groups. Its ^{1}H NMR spectrum showed typical signals for a β-sustituted furan ring at δ 6.5 (*brs*, H-14), 7.42 (*m*, H-15) and 7.27 (*m*, H-16), as well as signals corresponding to an olefinic β-proton of an α,β-unsaturated γ-lactone group at 6.93 (*brd*, H-3). The AB system at δ 4.03 (*dd*, H-19a) and 4.61 (*d*, H-19b) can be assigned to the oxymethylene protons of the lactone group (Seto et al., 1987; Esquivel et al., 1989; Maldonado et al., 1996; Maldonado and Ortega, 1997). Two olefinic protons at δ 6.23 (*ddd*, H-1) and 6.17 (*ddd*, H-2) were also present. The COLOC experiment showed cross peaks between C-18 (δ 169.3) and H-3 and H-19 that confirmed the presence of the α,β-unsaturated γ-lactone group. The *W*-coupling shown by the H-19 *pro* S indicated an α-axial configuration of the C-19 methylene group in agreement with *trans*-clerodanes having an axial methyl group at C-5 and an axial H-6 (Faini et al., 1987; Sánchez et al., 1995).

On the basis of its spectroscopic data and comparison with those of flabeloic acid, compound **5** was assigned to be 15,16-epoxy-*neo*-clerodan-1,3,13(16),14-tetraen-18,19-olide.

The close relationship between compound **5** and the secoclerodane **4** could be explained by considering that a conrotatory electrocyclic rearrangement caused by light on compound **5** generates the unusual diterpene **4**.

3. Experimental

3.1. General

CC: silica gel 60 (Merck), 0.063–0.200 mm; TLC: Kieselgel mit Fluoreszenz-Indicator UV_{254} (Macherey-Nagel), 0.25 mm; IR: in the 4000–225 cm^{-1} range with 32 scans using the KBr pellet technique. Spectral resolution: 4 cm^{-1}. (Nicolet Protégé 460 spectrometrer); ^{1}H NMR: $CDCl_3$ deuterated solvent, at 200.13 MHz relative to TMS at ($\delta = 0.00$) on a Bruker AC-200 spectrometer, 2D NMR spectra were measured as usual; EIMS: at 70 eV on GCQ Plus instrument.

3.2. Plant material

Aerial parts (2800 g) of *Baccharis flabellata* Hook. & Arn. var. *flabellata* (Asteraceae) were collected in February 1997 in "Cruz de Piedra" Dike, San Luis, Argentina. A voucher specimen (no. 434) is deposited in the herbarium of the University of San Luis.

3.3. Extraction procedure

A crude extract (372 g) of powdered air-dried aerial parts (2800 g) was obtained by extraction with acetone (×3) at room temp.

3.4. Isolation procedure

The acetone extract (372 g) was subjected to flash chromatography with *n*-hexane. The polarity of the solvent was gradually increased by addition of EtOAc. Six fractions were obtained.

Fraction I (30 g) obtained from 5% EtOAc was subjected to CC in silica gel eluting with a *n*-hexane:EtOAc mixture in increasing polarities to obtain epoxide **3** (62 mg).

Fraction II (eluted with 15% EtOAc, 34 g) was separated by flash chromatography and three subfractions were obtained. Subfraction 2 (20% EtOAc, 17.4 g) was further purified by CC on silica gel 60 and lactones **4** (311 mg) and **5** (64 mg) were obtained. From subfraction 3 (25% EtOAc, 2.231 g), flabeloic acid (849 mg) was purified by CC on silica gel 60, eluted with *n*-hexane:EtOAc.

Fraction III (40% EtOAc, 102.5 g) was separated by flash chromatography yielding flabeloic acid and oleanolic acid mixture (576 mg), and then purified by repeated CC (*n*-hexane:EtOAc).

3.5. 2,19;15,16-Diepoxy-neo-clerodan-3,13(16),14-trien-18-oic acid (3)

Colourless oil; $[\alpha]_D^{20} = +6.1^\circ$ ($CHCl_3$; *c* 0.26); IR ν_{max}^{KBr} cm^{-1}: 3585, 2955, 2865, 2351, 1716 (C=O), 1683, 1652,

1635, 1615 (C=C), 1435, 1361, 1254 (C-O), 1012; for ^{1}H and ^{13}C NMR spectral analysis, see Tables 1 and 2; HREIMS: *m/z*: 330.181297 (calc. 330.193110) [$C_{20}H_{26}O_4$]; EIMS (probe, 70 eV) *m/z* (rel. int.): 330 (1) [M^+], 312 (50), 288 (1), 271 (3), 248 (1), 231 (27), 218 (100), 217 (77), 203 (17), 190 (22), 189 (45), 164 (43), 95 (20), 81 (46).

3.6. 15,16-Epoxy-5,10-seco-clerodan-1(10),2,4,13(16),14-pentaen-18,19-olide (**4**)

Colourless oil; $[\alpha]_D^{20} = -135.7^\circ$ ($CHCl_3$; *c* 0.28); IR ν_{max}^{KBr} cm^{-1}: 2970, 2880, 1753, 1642, 1561, 1448, 1377, 1236, 1159, 1020; for ^{1}H and ^{13}C NMR spectral analysis, see Tables 1 and 2; HREIMS: *m/z*: 312.172494 (calc. 312.172545) [$C_{20}H_{24}O_3$]; EIMS (probe, 70 eV) *m/z* (rel. int.): 312 (7) [M^+], 297 (1), 295 (1), 284 (1), 270 (1), 253 (2), 231 (100), 218 (20), 217 (11), 189 (35), 174 (2), 173 (9), 162 (5), 161 (18), 95 (10), 82 (21), 81 (43).

3.7. 15,16-Epoxy-neo-clerodan-1,3,13(16),14-tetraen-18,19-olide (**5**)

Colourless oil; $[\alpha]_D^{20} = +6.1^\circ$ ($CHCl_3$; *c* 0.26); IR ν_{max}^{KBr} cm^{-1}: 3419, 3141, 3061, 2963, 2924, 2876, 1755, 1669, 1645, 1568, 1540, 1521, 1471, 1456, 1338, 1255, 1068, 1024; for ^{1}H and ^{13}C NMR spectral analysis, see Tables 1 and 2; HREIMS: *m/z*: 312.169475 (calc. 312.172545) [$C_{20}H_{24}O_3$]; EIMS (probe, 70 eV) *m/z* (rel. int.): 312 (1) [M^+], 297 (1), 270 (2), 268 (2), 253 (2), 231 (1), 218 (2), 217 (3), 203 (3), 189 (9), 176 (100), 175 (78), 163 (6), 162 (21), 161 (11), 149 (8), 135 (7), 95 (21), 81 (20), 67 (26).

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