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ENT-CLERODANE DERIVATIVES AND OTHER CONSTITUENTS FROM REPRESENTATIVES OF THE SUBGENUS *AGERATINA*

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Key Word Index—*Ageratina ixiocladon, A. pazcuarensis, A. prunellaefolia*; Compositae; diterpenes; *ent*-clerodanes; *p*-hydroxyacetophenone derivatives.

Abstract—*Ageratina ixiocladon* afforded several *p*-hydroxyacetophenone derivatives and diterpenes including six new *ent*-clerodanes. Two further species, which are also placed in the subgenus *Ageratina*, gave *p*-hydroxyacetophenone derivatives which seem to be characteristic for this group. The structure were elucidated by high field ^{1}H NMR techniques.

INTRODUCTION

Many species of the large genus *Ageratina* (Compositae, tribe Eupatorieae) have been studied chemically. In continuation of our investigations of this genus [1, 2] we have studied three species which have been placed in the subgenus *Ageratina* [3]. The results are discussed in this paper.

RESULTS AND DISCUSSION

Ageratina ixiocladon (Benth. ex Oersted) King et Robins. (= *Eupatorium ixiocladon*) grows in Costa Rica and Panama [4]. The roots contain large amounts of compound **11** [5] while the aerial parts afforded, in addition to known compounds (see Experimental), the *ent*-clerodanes **1–8**, **9** [6] and **10** [7] which were isolated as their methyl esters. Furthermore the chromenes **11** and **12** [1] were present.

The structure of **1** followed from the ^{1}H NMR spectrum of the corresponding methyl ester **1a** which was in part very close to that of a clerodane which only differed in the side chain [7]. The nature of the latter could be deduced from the characteristic ^{1}H NMR spectral data which also showed that a *Z*-configuration of the Δ^{13} bond was present. The ^{13}C NMR spectrum (see Experimental) also supported the structure. In the spectrum of **2a** the changed situation of the side chain followed from the corresponding ^{1}H NMR spectral signals which required a 13,14-dihydro derivative of **1a**. The configuration at C-13 was not determined.

The ^{1}H NMR spectra of **3a** and **4a** were similar. However, a pair of doublets between δ 2.3 and 3.1 differed typically. The couplings and the chemical shifts indicated the presence of epoxides. When the data were compared with those of **1a** it was obvious that we were dealing with the epimeric 4,18-epoxides. The observed NOEs in the case of **4a** showed that a β-epoxide was present. Thus a strong effect was observed between H-19 and H-18. NOEs between H-19 and H-3 axial, as well as between H-20, H-17 and H-19, were present and thus the stereochemistry was settled. In the ^{1}H NMR spectrum of **3a** a *W*-coupling between H-3 and H-18 indicated the presence of an α-epoxide. The ^{13}C NMR spectral data of **4a** also supported the proposed structure.

The ^{1}H NMR spectra of **5a** and **6a** clearly showed that the 13,14-dihydro derivatives of **3a** and **4a** were present. The ^{1}H NMR spectrum of **7a** again was close to that of **1a**, however, the exomethylene group at C-4 was replaced by a methyl carbinol as followed from the methyl singlet at δ 1.31 and the absence of exomethylene proton signals. As no NOE was observed between H-19 and H-18 a 4α-configuration was present. Clear effects between H-19 and H-20 as well as between H-17 and H-20 indicated the usual stereochemistry at C-5, C-8 and C-9.

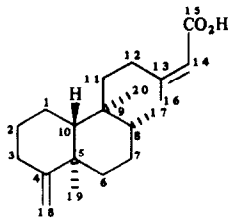
The ^{1}H NMR spectrum of **8a** and its molecular formula indicated the presence of a 2-keto derivative of **1a** with a Δ^3-bond. Accordingly, a pair of double doublets at δ 2.48 and 2.36 were visible while most of the remaining signals were similar to those of **1a**. The observed negative Cotton-effect of **8a** indicated that an *ent*-clerodane was present as the conformation of the A-ring followed from the coupling of H-10 [8]. Therefore all the diterpenes most likely are belonging to the *ent*-series.

The aerial parts of *A. pazcuasensis* (HBK) King et Robins. afforded the degraded *p*-hydroxyacetophenone derivatives **14** [9], **15** [10] and **16** [10] while those of *A. prunellaefolia* (HBK) King et Robins. gave **11**, **13** [1] and **14**.

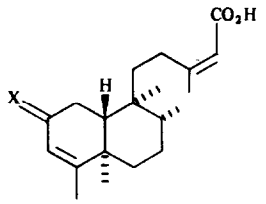
The absence of sesquiterpene lactones and the isolation of *p*-hydroxyacetophenone derivatives from these three species placed in the subgenus *Ageratina* again shows that these compounds are characteristic for this group. However, the relevance of the *ent*-clerodanes still is not clear. Further investigations may show whether these compounds are more widespread in this subgenus.

EXPERIMENTAL

The air-dried plant material was extracted and the extracts worked-up and separated as reported previously [8]. The extract of the roots (55 g) of *A. ixiocladon* (collected on the way to the vulcan Turrialba, Costa Rica, May 1987, voucher RMK 9696,

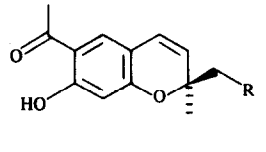


1
2 13.14 H
3 4α,18-epoxide
4 4β,18-epoxide
5 13.14 H, 4α,18-epoxide
6 13.14 H, 4β,18-epoxide
7 4.18 H, 4α OH

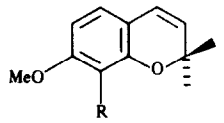


8 X = O
9 X = O, 13.14 H
10 X = H_2, 13.14 H

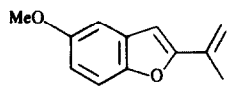
1a–10a are the corresponding methylesters



11 R = H
12 R = OH



13 R = H
14 R = OMe



15 **16** 2.3H

deposited in the National Herbarium, Washington, U.S.A.) gave by CC 200 mg **11**. The extract of the aerial parts (690 g) was separated first by CC into five fractions (Fr. 1: petrol; Fr. 2: Et_2O–petrol (1:3); Fr. 3: Et_2O–petrol (1:1); Fr. 4: Et_2O–petrol (2:1) and Fr. 5: Et_2O). TLC of fraction 1 gave 150 mg germacrene D and 150 mg γ-humulene. Fraction 2 contained a mixture of acids. After addition of CH_2N_2 in Et_2O TLC of 10% of the fraction (Et_2O–petrol, 1:2) gave 10 mg **11**, 10 mg spathulenol and 12 mg **1a** (R_f 0.91). Repeated CC of fraction 3 gave four fractions (Fr. 3/1–Fr. 3/4). After addition of CH_2N_2, 10% of fraction 3/1 gave by TLC (silica gel, $AgNO_3$ coated, Et_2O–petrol, 1:9) 15 mg **10a** and 30 mg **2a**. Repeated TLC of fraction 3/2 (one-tenth) gave 5 mg **4a** (R_f 0.63) and 5 mg (**3a** (R_f 0.60). After addition of CH_2N_2 fraction 3/3 gave a mixture of esters of which 5% was separated by HPLC (RP 8, MeOH–H_2O, 4:1, flow rate, *ca* 3 ml/min) affording 6 mg **7a** (R_t 16.5 min). TLC of fraction 3/4 gave 6 mg **12**. After esterification (CH_2N_2) one-sixth of fraction 4 was separated again by CC (silica gel, θ 30–60 μ, medium pressure) affording 50 mg **2a**, a mixture of 70 mg **5a** and **6a** as well as 400 mg **7a**. HPLC of 20% of the mixture of **5a**/**6a** (MeOH–H_2O, 17:3) gave 2.5 mg **5a** (R_t 13.1 min) and 6 mg **6a** (R_t 14.9 min). After addition of CH_2N_2 5% of fraction of 5 gave by HPLC (MeOH–H_2O, 4:1) 8 mg **9** (R_t 13.3 min) and 10 mg **8** (R_t 15.0 min). CC and TLC of the extract of 350 g aerial parts of *A. pazcuarensis* (voucher B. L. Turner 5393, deposited in the Herbarium of the University of Texas at Austin, U.S.A.) gave 3 mg germacrene D, 10 mg bicyclogermacrene, 8 mg α-humulene, 3 mg α-farnesene, 300 mg **14**, 30 mg **15** and 8 mg **16**.

CC and TLC of the extract of 100 g aerial parts of *A. prunellaefolia* (voucher B. L. Turner 15482, deposited in the Herbarium of the University of Texas at Austin, U.S.A.) gave 8 mg **11**, 12 mg **13** and 15 mg **14**.

ent-*Cleroda*-4(18),13*Z*-*dien*-15-*oic acid* (**1**). Isolated as its methyl ester **1a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3090, 1650 (C=CH_2), 1725 (C=CCO_2R); MS *m/z* (rel. int.): 318.256 $[M]^+$ (18) (calc. for $C_{21}H_{34}O_2$: 318.256), 303 $[M-Me]^+$ (6), 191 [M−side chain]$^+$ (82), 95 $[C_7H_{11}]^+$ (100); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.88 (*dddd*, H-2), 2.3 (*m*, H-3), 2.10 (*dddd*, H-3'), 1.47 (*m*, H-7), 1.52 (*m*, H-8), 1.12 (*dd*, H-10), 1.37 (*m*, H-11), 1.29 (*m*, H-11'), 2.50 (*dt*, H-12), 2.30 (*m*, H-12'), 5.59 (*br s*, H-14), 1.85 (*d*, H-16), 0.84 (*d*, H-17), 4.49 (*br s*, H-18), 1.04 (*s*, H-19), 0.72 (*s*, H-20), 3.75 (*s*, OMe); *J* [Hz]: 1,2 = 1', 2 = 2,3 = 2,3' = 2; 1,10 = 2.5; 1',10 = 12; 2,2' = 13; 2,3' = 2',3' = 3',18 = 3,3' = 15; 8,17 = 6; 11,12 = 12,12' = 12; 11',12 = 4.5; 14,16 = 1.5 (assigned by spin decoupling and NOE difference spectroscopy); ^{13}C NMR ($CDCl_3$, 100.6 MHz, C-1–C-20): δ 21.7, 33.1, 28.6, 161.2, 40.0, 37.3, 27.5, 36.6, 39.6, 48.5, 36.4, 27.3, 160.6, 115.4, 166.6, 25.3, 16.0, 102.5, 20.8, 18.0; OMe: 50.8 (assigned by 2D-techniques); $[\alpha]_D^{24}$ +14° ($CHCl_3$; *c* 0.19).

ent-*Clerod*-4(18)-*en*-15-*oic acid* (**2**). Isolated as its methyl ester **2a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3090, 1635 (C=CH_2), 1745 (CO_2R); MS *m/z* (rel. int.): 320.272 $[M]^+$ (6) (calc. for $C_{21}H_{36}O_2$: 320.272), 305 $[M-Me]^+$ (2), 289 $[M-OMe]^+$ (2), 191 [M−side chain]$^+$ (92), 95 $[C_7H_{11}]^+$ (100); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.30 (*m*, H-3), 2.11 (*br d*, H-3'), 1.05 (*dd*, H-10), 1.83 (*m*, H-13), 2.30 and 2.09 (*dd*, H-14), 0.91 (*d*, H-16), 0.78 (*d*, H-17), 4.49 (*br s*, H-18), 1.03 (*s*, H-19), 0.71 (*s*, H-20), 3.66 (*s*, OMe); *J* [Hz]: 1, 10 = 3; 1',10 = 11; 8,17 = 6; 13, 14 = 13,14' = 8; 13,16 = 7; 14,14' = 15; $[\alpha]_D^{24}$ +32° ($CHCl_3$; *c* 0.93).

4α,18-*Epoxy*-ent-*clerod*-13*Z*-*en*-15-*oic acid* (**3**). Isolated as its methyl ester **3a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1725 (C=CCO_2R); MS *m/z* (rel. int.): 334.251 $[M]^+$ (4) (calc. for $C_{21}H_{34}O_3$: 334.251), 319 $[M-Me]^+$ (4), 303 $[M-OMe]^+$ (8), 207 [M−side chain]$^+$ (66), 189 $[207-H_2O]^+$ (52), 177 (76), 109 (100), 95 (86), 69 (98), 55 (82); ^1H NMR ($CDCl_3$, 400 MHz as 1*a* except: δ 0.82 (*d*, H-17), 3.06 (*dd*, H-18), 2.39 (*d*, H-18'), 1.11 (*s*, H-19); *J* [Hz]: 3,18 = 2; 8,17 = 6.5; 18,18' = 4.5; $[\alpha]_D^{24}$ +3° ($CHCl_3$; *c* 0.23).

4β,18-*Epoxy*-ent-*clerod*-13*Z*-*en*-15-*oic acid* (**4**). Isolated as its methyl ester **4a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1725 (C=CCO_2R); MS *m/z* (rel. int.): see **4a**; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.40 (*m*, H-1), 1.0 (*m*, H-1'), 1.75 (*m*, H-2), 1.64 (*m*, H-2'), 2.15 (*ddd*, H-3), 1.0 (*m*, H-3'), 1.48 (*m*, H-8), 1.46 (*m*, H-11), 1.30 (*m*, H-11'), 2.54 (*dt*, H-12), 2.33 (*dt*, H-12'), 5.61 (*br s*, H-14), 1.87 (*d*, H-16), 0.83 (*d*, H-17), 2.77 (*d*, H-18), 2.33 (*d*, H-18'), 1.09 (*s*, H-19), 0.69 (*s*, H-20), 2.77 (*d*, H-18), 2.33 (*d*, H-18'), 1.09 (*s*, H-19), 0.69 (*s*, H-20), 3.67 (*s*, OMe); *J* [Hz]: 2,3 = 5; 2',3 = 3,3' = 14; 8,17 = 7; 11,12 = 11,12' = 18,18' = 4.5; 11',12 = 12,12' = 12; 13,16 = 1.5; ^{13}C NMR ($CDCl_3$, 67.9 MHz, C-1–C-20): 21.1, 24.2, 30.7, 65.4, 37.4, 30.5, 26.7, 35.9, 39.2, 45.3, 36.1, 27.4, 161.0, 115.5, 166.6, 25.4, 16.0, 49.9, 20.2, 17.8; OMe: 50.8; $[\alpha]_D^{24}$ +14° ($CHCl_3$; *c* 0.25).

4α,18-*Epoxy*-ent-*clerodane*-15-*oic acid* (**5**). Isolated as its methyl ester **5a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730 (CO_2R); MS *m/z* (rel. int.): 336.266 $[M]^+$ (14) (calc. for $C_{21}H_{36}O_3$: 336.266), 321 $[M-Me]^+$ (11), 305 $[M-OMe]^+$ (28), 207 [M−side chain]$^+$ (61), 95 (99), 55 (100); ^1H NMR ($CDCl_3$, 400 MHz) as **2a** except: δ 3.06 (*dd*, H-18), 2.40 (*d*, H-18'), 1.10 (*s*, H-19), 0.71 (*s*, H-20); *J* [Hz]: 3,18 = 2.5; 18,18' = 4.5; $[\alpha]_D^{24}$ +17° ($CHCl_3$; *c* 0.3).

4β,18-*Epoxy*-ent-*clerodane*-15-*oic acid* (**6**). Isolated as its methyl ester **6a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730 (CO_2R); MS *m/z* (rel. int.): 336.266 $[M]^+$ (66) (calc. for $C_{21}H_{36}O_3$: 336.266), 321 $[M-Me]^+$ (6), 305 $[M-OMe]^+$ (9), 207 [M−side chain]$^+$

(100), 95 (75); ^{1}H NMR ($CDCl_3$, 400 MHz) as **5a** except: 2.77 (*d*, H-18), 2.33 (*d*, H-18'), 1.07 (*s*, H-19), 0.68 (*s*, H-20); *J* [Hz]: 18,18' = 4.5; $[\alpha]_D^{24^\circ}$ +32° ($CHCl_3$; *c* 0.3).

4α-Hydroxy-ent-*clerod*-13*Z*-*en*-15-*oic acid* (**7**). Isolated as its methyl ester **7a**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1710 (C=CCO$_2$R); MS *m/z* (rel. int.): 336.266 [M]$^+$ (4) calc. for $C_{21}H_{36}O_3$: 336.266), 304 [M−MeOH]$^+$ (28), 209 [M−side chain]$^+$ (56), 191 (209−H_2O]$^+$ (100), 95 (72), 55 (62); ^{1}H NMR ($CDCl_3$, 400 MHz) as **1a** except: δ1.31 (*s*, H-18), 1.04 (*s*, H-19), 0.73 (*s*, H-20); $[\alpha]_D^{24^\circ}$ −0.3° ($CHCl_3$; *c* 0.32).

2-*Oxo*-ent-*cleroda*-3,13*Z*-*dien*-15-*oic acid* (**8**). Isolated as its methyl ester **8a**; colourless gum; 1720 (C=CCO$_2$R), 1660, 1620 (C=CC=O); MS *m/z* (rel. int.): 332.235 [M]$^+$ (8) (calc. for $C_{21}H_{32}O_3$: 332.235), 300 [M−MeOH]$^+$ (22), 285 [300−Me]$^+$ (34), 205 [M−side chain]$^+$ (100), 121 (90), 109 (78), 95 (98); ^{1}H NMR ($CDCl_3$, 400 MHz): δ2.48 (*dd*, H-1), 2.36 (*dd*, H-1'), 5.73 (*br s*, H-3), 1.61 (*ddq*, H-8), 1.96 (*dd*, H-10), 1.52 (*m*, H-11), 1.40 (*dt*, H-11'), 2.64 (*dt*, H-12), 2.24 (*dt*, H-12'), 5.62 (*br s*, H-14), 1.87 (*d*, H-16), 0.88 (*d*, H-17), 1.88 (*d*, H-18), 1.10 (*s*, H-19), 0.79 (*s*, H-20), 3.67(*s*, OMe); *J* [Hz]: 1,1' = 18; 1,10 = 4; 1',10 = 14; 3,18 = 1; 8,17 = 6.5; 11,11' = 11,12 = 12,12' = 12; 11,12' = 11',12' = 5; 14,16 = 1.5; $[\alpha]_D^{24^\circ}$ −6.5° ($CHCl_3$; *c* 0.25); CD (MeCN): $\Delta\varepsilon_{341}$ −0.87; $\Delta\varepsilon_{330}$ −0.91.

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