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COUMARIN AND TERPENOIDS FROM *PEREZIA ALAMANI* VAR. *OOLEPIS*

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Key Word Index—*Perezia alamani* var. *oolepis*; Compositae; roots; coumarin; sesquiterpenes; isoparvifoline; 8-hydroxypereflorine; diperezone; structural determination.

Abstract—Isoparvifolinone (**2a**), 8-hydroxypereflorine (**3a**) and diperezone (**5**) and the known sesquiterpenes cyperene and parvifoline (**1a**) were isolated from the roots of *Perezia alamani* var. *oolepis*. The structures of the new compounds were deduced from spectral data and tested by chemical correlation. Thus diperezone (**5**) was identical to the dimerization product of perezone (**4a**), isoparvifolinone (**2a**) was partially synthesized from parvifoline (**1a**) and 8-hydroxypereflorine (**3a**) was converted into the known 8-methoxypereflorine (**3b**).

INTRODUCTION

Previously we described the isolation and characterization of several sesquiterpenes from the roots of *Perezia carpholepis* [1]. Among others, cyperene, cyperone and parvifoline (**1a**) were found. We have now completed the study of another related plant, *P. alamani* var. *oolepis* from which cyperene, parvifoline (**1a**) and the three new compounds isoparvifolinone (**2a**), 8-hydroxypereflorin (**3a**) and diperezone (**5**) were isolated. The structural elucidation studies of these three new compounds are described in the present communication.

RESULTS AND DISCUSSION

Extensive chromatography of the hexane extracts of the roots of *P. alamani* var. *oolepis* gave cyperene [2] in 0.08% yield from the less polar fractions, followed by 0.2% of parvifoline [3] (**1a**). Both compounds were identified by direct comparison with samples isolated from *P. carpholepis*.

Further chromatography eluates provided a solid, mp 157–158°, that analysed for $C_{15}H_{18}O_2$. The nature of the two oxygen atoms was defined after inspection of the IR spectrum. Absorptions at 3600 and 3350 are attributed to a hydroxyl group and bands at 1660 and 1590 cm^{-1} correspond to a conjugated carbonyl group, further evident from the UV absorption at 3.37 nm (ϵ 9200). The phenolic nature of the hydroxyl group was deduced after inspection of the ^1H and ^{13}C NMR spectra, which in addition gave further structural information. The aromatic ring is evident from a ^{13}C oxygen bearing singlet at δ 155.5, three singlets at δ 143.7, 128.5 and 122.1 and two doublets at δ 133.4 and 111.3 and from two aromatic *para* distributed protons at δ 7.06 and 6.78, the former being broader due to benzylic coupling to an aromatic methyl group responsible for a 3H singlet at δ 2.25. The remaining sp^2 ^{13}C signals are ascribed to a carbonyl group at δ 205.4 which is in conjugation with a trisubstituted double bond responsible for a singlet at δ 135.6 and a doublet at δ 140.2. One of the substituents of the double bond is a methyl group responsible for a 3H doublet ($J = 1.7$ Hz) at δ 2.02, coupled to a vinyl proton that appears as a quartet ($J = 1.7$ Hz) with further unresolved long range couplings at δ 7.11, its chemical shift being indicative of its β-relation to the carbonyl group. The vinyl and aromatic methyl groups appear at δ 2.05 and 15.4 in the ^{13}C NMR spectrum. The remaining sp^3 signals are due to a benzyl substituted secondary methyl group responsible for a quartet at δ 19.4 and a doublet at δ 33.4 and a 1,2-disubstituted ethane residue giving two triplets at δ 42.2 and 39.0 in the ^{13}C NMR spectrum. The secondary methyl group is further evident from a 3H doublet ($J = 7$ Hz) at δ 1.27 which is coupled to a 1H methine multiplet at δ 2.96. The combined spectral data for the natural product, bearing in mind those data of parvifoline (**1a**), allow postulation of structure **2a**. Definitive evidence for the structure of isoparvifolinone as **2a** was obtained by its partial synthesis from parvifoline (**1a**).

Treatment of parvifoline acetate (**1b**) with boiling acetic acid in the presence of a catalytic amount of zinc caused isomerization of the double bond, which migrates to conjugate with the aromatic ring. Isoparvifoline acetate (**2b**) showed UV absorption bands at 226 ($\epsilon = 8900$) and 247 nm ($\epsilon = 10\,200$) and the presence of a phenolic acetate at 1760 cm^{-1} in the IR spectrum. Its ^1H NMR spectrum is in full agreement with structure **2b**. The aromatic protons appear at δ 6.96 (H-4) and 6.86 (H-1), the aromatic methyl at δ 2.11, the benzyl substituted secondary methyl group signals as a 3H doublet at δ 1.24 and a broad multiplet at δ 3.11, and the acetyl methyl as a 3H singlet at

δ 2.28. The isomerized double bond signals are responsible for a 1H quartet ($J = 1.2$ Hz) at 6.19 and a 3H doublet ($J = 1.2$ Hz) at δ 1.86.

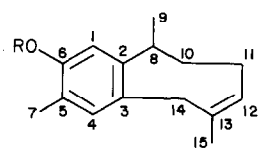
Chromium trioxide oxidation of the allylic methylene group of **2b** to a carbonyl group completed the creation of the chromophore and removal of the protecting acetyl group gave a sample identical to naturally occurring isoparvifolinone (**2a**).

Fractions eluted with ethyl acetate from the chromatography of the extracts, gave a solid mp 230–232° that analysed for $C_{11}H_{10}O_4$. The UV spectrum showed absorptions at 208, 256 and 289 nm (ϵ = 31 600, 11 500 and 12 900) and the IR spectrum showed a hydroxyl group at 3550, a carbonyl at 1730 and double bond bands at 1620 and 1590 cm^{-1}. The elemental composition and complex UV spectrum are indicative of a highly aromatic structure. Particularly informative was the ^1H NMR spectrum measured in DMSO-d_6. An hydroxyl signal as a broad peak at 9.70, two *ortho* distributed aromatic protons as doublets ($J = 8$ Hz) at 6.93 and 6.83, the latter being broader due to benzylic coupling to an aromatic methyl group at δ 2.43, a methoxyl singlet at 3.93 and especially a 1H singlet at δ 5.70, strongly suggested a coumarin structure having an oxygen atom at C-4 and the remaining groups on the aromatic ring. Cogent evidence for a 5-methyl coumarin with oxygen atoms at C-4 and C-8 was adduced from the totally proton-coupled and totally proton-decoupled ^{13}C NMR spectra of the natural product, also obtained from DMSO-d_6 solutions. Quaternary carbon signals at 168.9, 161.1, 143.0 (2C), 125.3 and 114.3 are ascribed[4] to C-4, C-2, C-8, C-9a, C-5 and C-4a, respectively. Doublets with no further long-range couplings at 117.7 ($^1J = 156$ Hz) and at 89.7 ($^1J = 165$ Hz) of quartets ($J = 8$ Hz) at δ 126.7 was due to C-6. The remaining ^{13}C signals were a methoxyl quartet ($^1J = 146$ Hz) at 56.5 and a methyl group at δ 22.3 which appeared as a quartet ($^1J = 125$ Hz) of doublets ($^3J = 6$ Hz).

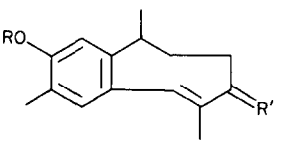
Chemical evidence for a 5-methylcoumarin having oxygen atoms at C-5 and C-8 was obtained after methylation of the natural product which yielded a sample identical to 8-methoxypereflorin[5] (**3b**) previously isolated from the closely related *P. multiflora*[6] that grows in South America. Therefore, in order to complete the structural elucidation of the natural product it remained only to define whether the oxygen atom at C-5 or that at C-8 was the methylated one. This was achieved by comparison of the ^{13}C NMR chemical shifts of the natural product to those of 8-methoxypereflorin (**3b**) measured in the same solvent. The data obtained from **3b** are summarized in the Experimental and the comparison reveals that the new natural product is 8-hydroxypereflorine (**3a**), since the chemical shifts of C-3 and C-4 are invariant on going from **3a** to **3b**, while those from C-7 and C-8 are shifted as expected, in accordance with their chemical difference. Furthermore, the other possibility, 4-hydroxy-5-methyl-8-methoxy coumarin was prepared by synthesis[7]; data differ from those of **3a**.

The last compound isolated from the chromatography of the extracts of *P. alamani* var. *oolepix* was obtained from chloroform eluates as a red viscous oil. Its ^1H NMR spectrum was very similar to that of hydroxyperezone[8] (**4b**), a natural product isolated from the very closely related *P. alamani* var. *adnata*[9]. The only difference between the spectra was found in the low field region. In the case of **4b**, two labile protons appeared as a singlet at δ 7.76, while in the new compound the presence of only one labile proton at δ 7.07 was apparent. In contrast, the ^{13}C NMR spectrum[10] of the new product was very similar to that of perezone (**4a**). The main difference occurred at the C-6 signals, which in **4a** appeared as a doublet at δ 135.6 and in the new compound as a singlet at δ 138.1. The combined NMR data were interpreted in terms of the perezone dimer **5**. The ^1H and ^{13}C data of **5**, the ^1H data of hydroxyperezone (**4b**) and the ^{13}C shifts of perezone (**4a**) are summarized in the Experimental for comparison purposes.

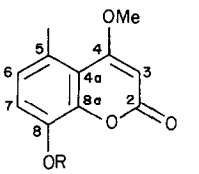
Convincing evidence for the structure of diperezone (**5**) was provided by the $[M]^+$ at *m/z* 494 in the mass spectrum. Other important fragments are given in the Experimental. Definitive structural proof



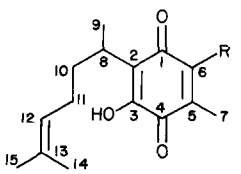
1a R=H
1b R=Ac



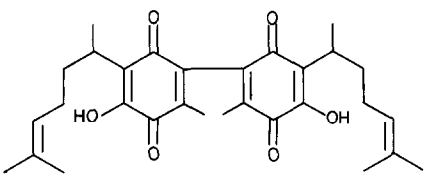
2a R=H, R'=O
2b R=Ac, R'=H_2



3a R=H
3b R=Me



4a R=H
4b R=OH



5

was obtained by direct comparison with a sample prepared by boron trifluoride catalysed dimerization[11] of perezone (**4a**).

From the chemotaxonomic point of view, it is interesting that *P. alamani* var. *adnata* contains hydroxyperezone (**4b**) which was not detected in *P. alamani* var. *oolepix*. These two plants belong to the section[12] *Acourtia* of the genus *Perezia* which is abundant in North America. In contrast *P. multiflora* belongs to the section[13] *Perezia* of the same genus, which is abundant in South America and also contains coumarins. The isolation of 8-hydroxypereflorine (**3a**) from a North American *Perezia* is the first chemical relationship found between the two sections of this genus.

EXPERIMENTAL

Mps are uncorr. IR spectra were determined in $CHCl_3$ soln, UV spectra in 95% EtOH, rotations in $CHCl_3$ and MS at 70 eV. ^1H NMR spectra were measured in $CDCl_3$ solns (unless otherwise specified) using TMS as int. standard. The microanalytical determinations were performed in the Alfred Bernhardt Laboratories, West Germany.

Extraction. Roots of *P. alamani* var. *oolepis* (Bartlett) were collected at Santiaguito, Michoacan in August 1978. Voucher samples are deposited at the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City where Professor J. Rzedowski kindly identified the material. The dried and ground roots (1.25 kg) were extracted twice with hexane (5 l.) under reflux for 6 hr. The combined extracts were evaporated to dryness (47 g) and chromatographed over Si gel (500 g). Fractions eluted with hexane gave 1.73 g cyperene and those eluted with hexane–C_6H_6 mixtures gave parvifoline (**1a**). Both compounds were identified by direct comparison with samples isolated from *P. carpholepis*[1]. Further chromatography eluates gave three additional main fractions: A (C_6H_6–$CHCl_3$; 3:1 to 1:1), B ($CHCl_3$) and C (EtOAc).

Isoparvifolinone (**2a**). Fraction A of the chromatography yielded 137 mg of the title compound, mp 153–156°. The analytical sample, obtained after re-crystallization from $CHCl_3$–hexane, had a mp 157–158°; $[\alpha]_D$ +854° (*c* 1); UV λ_{max} nm: 208, 235 and 337 (ϵ = 25 000, 22 500 and 9200); IR ν_{max} cm^{-1}: 3600 and 3350 (hydroxyl), 1660 (α,β-unsaturated carbonyl) and 1590 (C=C); ^1H NMR (100 MHz): 7.11 (*q* with further unresolved couplings, J_q = 1.7 Hz, 1H) H-14, 7.06 (*br s*, 1H) H-4, 6.78 (*s*, 1H) H-1, 5.55 (*s*, 1H exchangeable on addition of D_2O) OH, 2.96 (*br m*, 1H) H-8, 2.25 (*s*, 3H) aromatic Me, 2.02 (*d*, *J* = 1.7 Hz, 3H) vinyl Me and 1.27 (*d*, *J* = 7 Hz, 3H) sec Me); ^{13}C NMR: 111.3 (C-1), 143.7 (C-2), 128.5 (C-3), 133.4 (C-4), 122.1 (C-5), 155.5 (C-6), 15.4 (C-7), 33.4 (C-8), 19.4 (C-9), 42.2 (C-10), 39.0 (C-11), 205.4 (C-12), 135.6 (C-13), 140.2 (C-14), 20.5 (C-15). Anal. calc. for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88; O, 13.89%. Found: C, 78.14; H, 7.76; O, 13.78%.

Isoparvifoline acetate (**2b**). A soln of 500 mg **1b** in 5 ml HOAc was refluxed in the presence of 10 mg Zn dust for 4 hr. The reaction mixture was diluted with H_2O and extracted twice with Et_2O. The organic layer was washed with dil. $NaHCO_3$ and with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residue was chromatographed over 6 g Si gel. The fractions eluted with C_6H_6–$CHCl_3$ (1:1) were combined yielding 370 mg of the title compound as a viscous oil that showed $[\alpha]_D$ +209° (*c* 1); UV λ_{max} nm: 226 and 247 (ϵ = 8900 and 10 200); IR ν_{max} cm^{-1}: 1760 (acetate) and 1260 (C–C double bond); ^1H NMR (100 MHz): 6.96 (*br s*, 1H) H-4, 6.86 (*s*, 1H) H-1, 6.19 (*q*, *J* = 1.2 Hz, 1H) H-14, 3.11 (*br m* 1H) H-8, 2.28 (*s*, 3H) acetate, 2.11 (*s*, 3H) aromatic Me, 1.86 (*d*, *J* = 1.2 Hz, 3H) vinyl Me and 1.24 (*d*, *J* = 7 Hz, 3H) sec Me.

Isoparvifolinone (**2a**) *from isoparvifoline acetate* (**2b**). A soln of 300 mg **2b** in 5 ml HOAc was stirred in the presence of 500 mg CrO_3 at room temp. for 5 hr. The reaction mixture was diluted with ice–H_2O and extracted twice with EtOAc. The organic layer was washed with diluted $NaHCO_3$ and with H_2O, dried (Na_2SO_4), filtered and evaporated. The residue was dissolved in MeOH and hydrolysed in the presence of 250 mg KOH under reflux for 30 min. After usual work-up, the residue was chromatographed over 5 g Si gel. The fractions eluted with $CHCl_3$ gave 90 mg **2a**, mp 157–158° which was identical to a sample isolated from the roots.

Diperezone (**5**). Fraction B of the original chromatography was re-chromatographed over Si gel, yielding the title compound (100 mg) as a red viscous oil, $[\alpha]_{589}$ 0°, $[\alpha]_{546}$ +13° (*c* 1); UV λ_{max} nm: 209 and 274 (ϵ = 15200 and 10000); IR ν_{max} cm^{-1}: 3400 (hydroxyl), 1670 (carbonyl) and 1630 (C=C); ^1H NMR (100 MHz): 7.07 (*s*, 1H, exchangeable with D_2O) OH, 5.09 (*t* with further unresolved couplings, J_t = 7 Hz, 1H vinyl proton, 3.07 (*sextet*, *J* = 7 Hz, 1H) H-8, 1.88 (*s*, 3H) quinone Me, 1.66 and 1.56 (2*s*, 3H each) isopropylidene Mes and 1.21 (*d*, *J* = 7 Hz, 3H) secondary Me. ^{13}C NMR: 184.7 (C-1), 124.8 (C-2), 150.9 (C-3), 183.0 (C-4), 140.1 (C-5), 138.1 (C-6), 12.8 (C-7), 29.6 (C-8), 18.2 (C-9), 34.2 (C-10), 26.6 (C-11), 124.2 (C-12), 131.1 (C-13), 17.5 (C-14) and 25.6 (C-15); MS (70 eV) *m/z*: 494 $[M]^+$ 412 [M − 4-methyl-1, 3-pentadiene]$^+$ and 330 [M − 2 × (4-methyl-1, 3-pentadiene)]$^+$. For comparison purposes, the relevant data for **4a** and **4b** are given below. The compound is identical to the dimerization[11] product (**5**) of perezone (**4a**). Perezone (**4a**). ^{13}C NMR: 187.2 (C-1), 124.4 (C-2), 151.0 (C-3), 184.0 (C-4), 140.4 (C-5), 135.6 (C-6), 14.6 (C-7), 29.4 (C-8), 18.3 (C-9), 34.2 (C-10), 26.8 (C-11), 124.4 (C-12), 131.2 (C-13), 17.6 (C-14) and 25.9 (C-15). Hydroxyperezone (**4b**). ^1H NMR (100 MHz): 7.76 (*s*, 2H exchangeable with D_2O) 2 OH, 5.08 (*t* with further unresolved couplings, J_t = 7 Hz, 1H) vinyl proton, 3.05 (*sextet*, *J* = 7 Hz, 1H) H-8, 1.93 (*s*, 3H) quinone methyl, 1.64 and 1.53 (2*s*, 3H each) isopropylidene Mes and 1.21 (*d*, *J* = 7 Hz, 3H) secondary Me.

8-Hydroxypereflorine (**3a**). Fraction C of the original chromatography yielded 152 mg of the title compound, mp 230–232°. The analytical sample, obtained after recrystallization from $CHCl_3$ showed mp 238–240°; UV λ_{max} cm^{-1}: 3550 (hydroxyl), 1730 (carbonyl) and 1620 and 1590 (C=C). ^1H NMR (DMSO-d_6, 60 MHz): 9.70 (*br*, 1H) OH, 6.93 and 6.83 (2*d*, *J* = 8 Hz, 1H each) H-6 and H-7, 5.70 (*s*, 1H) H-3, 3.93 (*s*, 3H) OMe and 2.43 (*s*, 3H) aromatic Me. ^{13}C NMR (DMSO-d_6): 161.1 (C-2), 89.7 (*d*, ^1J = 168 Hz, C-3), 168.9 (C-4), 114.3 (C-4a)c 125.3 (C-5), 126.7 (*dq*, ^1Jd = 165 Hz, ^3Jq = 8 Hz, C-6), 117.7 (*d*, ^1J = 156 Hz, C-7), 143.0 (C-8), 143.0 (C-8a), 56.5 (*q*, ^1J = 146 Hz, OMe) and 22.3 (*qd*, ^1Jq = 125 Hz, ^3Jd = 6 Hz, aromatic Me). Anal. calc. for $C_{11}H_{10}O_4$: C, 64.08; H, 4.69; O, 31.04%. Found: C, 63.90; H, 4.91; O, 31.18%.

8-Methoxypereflorine (**3b**). A soln of **3a** (125 mg) in 10 ml Me_2CO was refluxed in the presence of 1.5 ml Me_2SO_4 and 1 g dry K_2CO_3 for 2.5 hr. The soln was filtered and the solvent evaporated. The residue was diluted with hexane and chromatographed over Si gel. The crystalline fractions eluted with $CHCl_3$ were combined and re-cyrstallized from $CHCl_3$–hexane to yield 80 mg of the title compound, mp 154–155°, identical to 8-methoxypereflorine (**3b**) isolated from *P. multiflora*[6]. ^{13}C NMR (DMSO-d_6): 160.8 (C-2), 90.0 (C-3), 168.7 (C-4), 114.2 (C-4a), 126.6 (C-5), 126.4 (C-6),

113.7 (C-7), 145.0 (C-8), 143.4 (C-8a), 56.2 and 55.9 (2 OMe) and 22.3 (Me).

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REFERENCES

1. Joseph Nathan, P., Hernández, J. D. , Román, L. U., García, G., E. and Mendoza, V. (1982), *Phytochemistry* 21, 669.
2. Trevedi, B., Molt, O. and Sorm, F. (1964), *Tetrahedron Letters*, 1197.
3. Bohlmann, F. and Zdero, C. (1977), *Chem. Ber.* **110**, 468.
4. Günther, H., Prestien, J. and Joseph-Nathan, P. (1975) *Org. Magn. Resonance* 7, 339.
5. Bohlmann, F. and Zdero, C. (1977) *Phytochemistry* **16**, 239.
6. Joseph-Nathan, P., Hidalgo, J. and Abramo-Bruno, D. (1978) *Phytochemistry* **17**, 583.
7. Bohlmann, F. and Wienhold, C. (1979), *Chem. Ber.* **112**, 2394.
8. Joseph-Nathan, P., González, M. P., García, G., E., Barios, H. and Walls, F. (1974) *Tetrahedron* **30**, 3461.
9. García, T., Domínguez, E. and Romo, J. (1965), *Bol. Inst. Quim. Univ. Nat. Auton. Méx.* **17**, 16.
10. Joseph-Nathan, P., Mejía, G. and Abramo-Bruno, D. (1979) *J. Am. Chem. Soc.* **101**, 1289.
11. Sánchez, I. H., Yañez, R., Enríquez, R. and Joseph-Nathan, P. (1981) *J. Org. Chem.* **46**, 2818.
12. Bacigalupi, R. (1931). *A Monograph of the Genus* Perezia *Section* Acourtia. The Gray Herbarium of Harvard, Cambridge.
13. Vuilleumier, B. S. (1970) *The Systematics and Evolution of* Perezia *Sect.* Perezia. The Gray Herbarium of Harvard, Cambridge.