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SESQUITERPENES FROM *PEREZIA CARPHOLEPIS*

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Key Word Index—*Perezia carpholepis*; Compositae; roots; sesquiterpenes; curcuquinol monoisovalerate.

Abstract—The previously unknown curcuquinol monoisovalerate and the known sesquiterpenes cyperene, cyperone, parvifoline, perezone, and α- and β-pipitzol were found in the roots of *Perezia carpholepis*. The structure of the new sesquiterpene was deduced from spectral data and by transformation into curcuquinone. Parvifoline and its derived acetate, previously thought to be oily materials, were characterized as crystalline compounds.

INTRODUCTION

In a continuation of our studies on the constituents of the genus *Perezia* [1], we have isolated and characterized several sesquiterpenes from *P. carpholepis*.

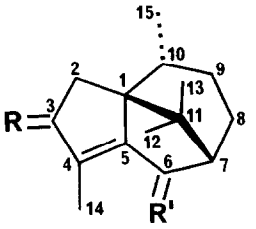
RESULTS AND DISCUSSION

Perezone [2] and the pipitzols [3] were crystallized from the hexane extract of the roots (3 and 0.1% of the dry wt respectively) and identified by direct comparison with authentic samples.

The mother liquors left after the crystallization were chromatographed repeatedly. The less polar fractions gave a colourless oil (0.3% yield), the ^{13}C NMR spectrum of which showed signals due to a tetrasubstituted double bond (δ 142.0 and 127.5), two quaternary carbons (δ 65.6 and 41.3), two CH carbons (δ 48.7 and 35.3), five methylene carbons (δ 42.3, 28.3, 27.8 and 27.5) and four methyl carbons (δ 26.1, 19.3, 17.9 and 14.1). The ^1H NMR spectrum contained signals due to two methyl groups (δ 0.77 and 0.95), a secondary methyl group ($J = 7$ Hz, δ 0.81) and a vinylic methyl group (δ 1.62). These data suggested a tricyclic monounsaturated sesquiterpene structure. The ^1H NMR spectrum was found to be in agreement with that of cyperene (**1a**), although the original assignments [4] of the methyl signals are incorrect. Direct comparison of our product with a commerical sample of cyperene (**1a**) established definitive identity. A second oily compound showed IR absorptions at 1700 and 1660 cm^{-1} indicative of an α,β-unsaturated ketone; confirmation of the presence of this functionality was provided by the UV spectrum (λ_{max} 246 nm, ϵ 10 000). The ^1H NMR spectrum resembled that of cyperene (**1a**), although the gemdimethyl signals now appeared at δ 0.76 and 1.10, the secondary methyl signal ($J = 7$ Hz) at δ 0.62 and the vinyl methyl signals as a triplet ($J = 2$ Hz) at δ 1.67. The combined spectral data suggested this compound was cyperenone (**1b**) [5]. This was confirmed by direct comparison with a sample prepared by chromium trioxide oxidation of cyperene (**1a**). The derived 2,4-dinitrophenylhydrazones were also identical. The isolation of cyperene-type compounds from the genus *Perezia* is interesting, since prior to this study the only known tricyclic sesquiterpenes were the cedrene derivatives [6].

Further chromatographic eluants gave a crystalline compound mp 89–90°, $[\alpha]_D - 173°$, that analysed for $C_{15}H_{20}O$. The sole oxygen atom was present in a phenolic group, since the IR spectra of the natural product and of the derived crystalline acetate showed absorptions at 3610 and at 1765 cm^{-1} respectively, although the natural product gave a negative $FeCl_3$ test. A literature search revealed that no compound with such physical and spectral data had been described. The structure of this compound was established as **2a** from its spectral data (summarized in the Experimental), and the spectral data of its acetate (**2b**) and the SeO_2 oxidation product **2c**. Once the molecular constitution was established, we found that the same structure (**2a**) had been assigned to an oily constituent of *Coreopsis parvifolia* [8] that gave a positive $FeCl_3$ test and also yielded an oily acetate, in contrast to our crystalline samples of **2a** and **2b**, the former giving in our hands a negative $FeCl_3$ test. However, ^1H NMR spectral measurements (270 MHz), revealed that our crystalline compounds had the same molecular constitution as **2a** from *C. parvifolia* and its derived acetate, the difference in physical data was attributed to impure samples being handled by the German group. In any event, our results further support the structure of parvifoline (**2a**).

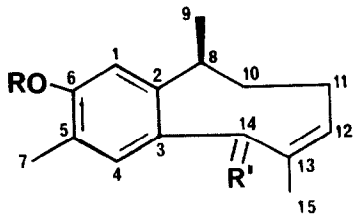
The last compound isolated from the roots was an oily material that showed IR absorptions due to a hydroxyl group (3600 cm^{-1}) and to a phenyl ester (1760 cm^{-1}). The nature of the esterifying residue was easily established as an isovaleryl group from the ^1H NMR doublet at δ 0.93, the $-OCOCH_2-$ signal at δ



1a R = H_2; R' = H_2

1b R = O ; R' = H_2

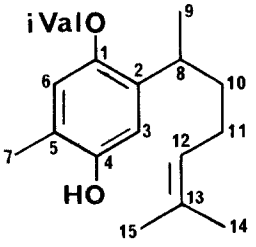
1c R = H_2; R' = O



2a R = H; R' = H_2

2b R = Ac; R' = H_2

2c R = H; R' = O



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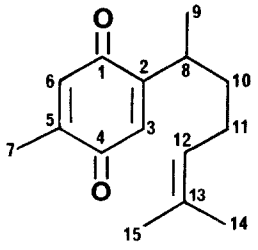

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2.36 and the ^{13}C NMR peaks at δ 172.2 (*s*), 43.3 (*t*), 31.9 (*d*) and 22.5 (*q*) [9]. Furthermore, in the mass spectrum, the molecular ion at *m/z* 318 defined the elemental composition as $C_{20}H_{30}O_3$ and a peak at *m/z* 234 was in agreement with $[M-C_5H_8O]^+$. The ^1H NMR spectrum showed that the remaining 15 carbon atoms formed a curcuquinol residue. Thus two *para* distributed aromatic protons appeared as singlets at δ 6.58 and 6.70, an aromatic methyl group appeared as a singlet at δ 2.08, a D_2O interchangeable hydroxyl signal appeared at δ 5.88 and characteristic signals [2] due to a $-CH(Me)CH_2CH_2CH=C(Me)_2$ fragment, were evident. The ^{13}C NMR values (Experimental) were also in full agreement with a curcuquinol skeleton. Chemical evidence to support the structure, was obtained by alkaline hydrolysis of the natural product. This yielded isovaleric acid which was characterized as its derived anilide by comparison with an authentic sample. The other fragment obtained from the hydrolysis, spontaneously oxidized during the reaction, yielding a yellow liquid whose ^1H NMR, IR and UV spectra were in agreement with those of curcuquinone (**4**), isolated recently [10] from the coral *Pseudoterogorgia rigida*. Direct comparison with a sample of curcuquinone (**4**) obtained by total synthesis [15] confirmed its identity. The preceding combined spectral and chemical evidence, allowed postulation of structure **3**, or alternately the isomer, in which the ester group is *ortho* to the methyl group. A distinction between these two alternatives was not possible from shift reagent induced shifts, but was achieved by detailed interpretation of the six ^{13}C NMR signals of the aromatic ring of the natural product. The ring signals for thymol and for isothymol had already been assigned [11]. Introduction of an OCOR group in a benzene ring will shift [12] the *ipso*, *ortho*, *meta* and *para* carbons by +22.4, −7.1, +0.4 and −3.2 ppm, respectively. This allows prediction of the chemical shifts of the ring carbons of both possible monoesterified thymoquinols. The experimental chemical shift for the monoisovaleryl curcuquinol were compared with the estimated values for the thymoquinol derivatives. Agreement was found only when the ester is placed *ortho* to the isopropyl group. The details of the calculation are given in the Experimental. This completed the structural elucidation of the curcuquinol monoisovalerate as **3**.

EXPERIMENTAL

Mps are uncorr. The microanalytical determinations were performed in the Alfred Bernhardt Laboratories, W. Germany.

Extraction of P. carpholepis (*A. Gray*). The roots were collected at Michac, Puebla, in Aug. 1978 and voucher samples were deposited at Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, where Prof. J. Rzedowski kindly classified the material. The dried and ground roots (1.25 kg) were extracted (×2) with hexane (5 l.) under reflux for 6 hr. The combined extracts were evaporated to a small vol. and left overnight at 4°. The

crystalline product was decanted. Recrystallization (Et_2O) gave 1.02 g of a mixture [3] of α- and β-pipitzol, identified by direct comparison with an authentic specimen. The mother liquors left from the isolation of the pipitzols, were crystallized from hexane, to give perezone (31 g), identified with an authentic sample [2].

The mother liquors of the original extract were evaporated to dryness (50 g) and chromatographed over Alcoa F-20 alumina (500 g); four main fractions were separated: A(C_6H_{14}), B(C_6H_{14}–C_6H_6, 9:1 to C_6H_6–$CHCl_3$, 1:3), C($CHCl_3$) and D(EtOAc–$CHCl_3$, 1:1).

Cyperene (**1a**). Fraction A was purified by distillation at 103–105° (5 mm Hg) to give 4 ml of a colorless oil, $[\alpha]_D$ − 22°(*c*, 2.27), ^{1}H NMR (60 MHz, $CDCl_3$, TMS int. standard): δ 1.62 [*s*(*br*), 3H, vinyl methyl) 0.81 (*d*, J = 7 Hz, 3H, secondary Me) and 0.95 and 0.77 (2*s*, 3H each, *gem*-dimethyl); ^{13}C NMR ($CDCl_2$, TMS int. standard): δ 142.0 (*s*, C-4), 127.5 (*s*, C-5.5 (*s*, C-1), 48.7 (*d*, C-7), 42.3 (*t*, C-6), 41.3 (*s*, C-11), 35.3 (*d*, C-10), 28.3, 27.8 and 27.5 (3*t*, C-2, C-8 and C-9), 26.3 (*t*, *C*-3), 26.1 and 17.9 (2*q*, *gem*-dimethyl), 19.3 (*q*, vinyl methyl) and 14.1 (*q*, secondary methyl).

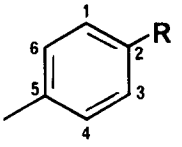
Cyperenone (**1b**). Fraction B was rechromatographed several times over SiO_2, to give 40 mg **1b**, as a colorless oil, $[\alpha]_D$ + 14.6° ($CHCl_3$; *c*, 5.49); UV $\lambda_{max}^{95\%EtOH}$ 246 nm (ϵ 10 000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 and 1660 (α, β-unsaturated ketone); ^{1}H NMR (60 MHz, $CDCl_3$, TMS int. standard): δ 1.67 (*t*, J = 2Hz, 3H, vinyl methyl), 1.10 and 0.76 (2*s*, 3H each, *gem*-dimethyl) and 0.62 (*d*, J = 7 Hz, 3H, secondary methyl); identical to a sample of cyperenone (**1b**) obtained by CrO_3 oxidation [5] of cyperene (**1a**); 2,4-dinitrophenylhydrazones showed mp 226–228° which was not depressed on admixture.

Parvifoline (**2a**). Fraction C was rechromatographed over SiO_2. The crystalline fractions were combined and recrystallized from Me_2CO–hexane to yield 60 mg of white needles, mp 85–86°. The analytical sample, obtained by recrystallization from the same solvents showed mp 89–90°; (−) $FeCl_3$ test; $[\alpha]_{589}$ − 173°, $[\alpha]_{578}$ − 186°, $[\alpha]_{546}$ − 210°, $[\alpha]_{436}$ − 374°, $[\alpha]_{365}$ − 374° ($CHCl_3$; *c*, 1.73); UV $\lambda_{max}^{95\%EtOH}$ 224 and 289 nm (ϵ, 12 000 and 7500); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3610 and 3350 (hydroxyl) and 1620 (C–C double bond); ^{1}H NMR (100 MHz $CDCl_3$, TMS int. standard): δ 6.91 [*s*(*br*), 1H, H-4], 6.62 (*s*, 1H, H-1), 5.36 (*t* with further unresolved couplings, J_t = 7 Hz, 1H, H-12), 4.62 (*s*, 1H, lost on addition of D_2O, OH), 3.51 and 3.02 (2*d*, J = 18 Hz, 1H each, H-14 and H-14′), 3.18 [multiplet (*br*) 1H, H-8], 2.19 (*s*, 3H, aromatic methyl) 1.74 [*s*(*br*), 3H, vinyl methyl] 1.30 (*d*, J = 7 Hz, 3H, secondary methyl), the remaining four protons (H-10, H-10′, H-11 and H-11′) were in the 1.6–2.0 (3H) region and at 1.1 (1H); ^{13}C NMR ($CDCl_3$, TMS int. standard) δ 111.3 (C-1), 143.9 (C-2), 130.7 (C-3), 131.8 (C-4), 120.4 (C-5), 152.5 (C-6), 15.3 (C-7), 33.1 (C-8), 19.2 (C-9), 39.9 (C-10), 23.8 (C-11), 123.3 (C-12), 137.5 (C-13), 41.6 (C-14) and 26.3 (C-15); ^{1}H NMR (60 MHz) Eu(fod)$_3$-induced shifts measured using a previously developed [1] experimental sequence (mol Eu(fod)$_3$/mol substrate): H-1, 1.96; H-4, 0.63; aromatic methyl, 1.33 and isolated CH_2, 0.29 ppm. (Anal. calc. for $C_{15}H_{20}O$: C, 83.29; H, 9.32; O, 7.40%. Found: C, 83.09; H, 9.24; O, 7.25%).

Parvifoline acetate (**2b**). Acetylation of 100 mg parvifoline (**2a**) in pyridine (1 ml) with Ac_2O (1 ml) on the steam-bath for 2 hr, yielded after work-up and oily residue. Chromatography over SiO_2 (3 g) gave 75 mg **2b**, mp 52–55° from the fractions eluted with hexane—C_6H_6 (9:1). The analytical sample, obtained after recrystallization from Me_2CO–hexane showed mp 59–61°, $[\alpha]_D$ − 98° ($CHCl_3$); UV $\lambda_{max}^{95\%}$ EtOH 224 and 280 nm (ϵ, 14 500 and 8000); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1765 (acetate) and 1650 and 1520 (C–C double bonds); ^{1}H NMR (100 MHz, $CDCl_3$, TMS int. standard): δ 7.02 [*s*(*br*), 1H, H-4], 6.80 (*s*, 1H, H-1), 5.38 (*t* with further unresolved couplings, J_t = 7Hz, 1H, H-12), 3.54 and 3.10 (2*d*, J = 18 Hz, 1H each, H-14 and H-14′), 3.21 [multiplet (*br*), 1H, H-8], 2.17 (*s*, 3H, acetate), 2.11 (*s*, H, aromatic Me), 1.76 [*s*(*br*), 3H, vinyl methyl), 1.31 (*d*, J = 7 Hz, 3H, secondary methyl), the remaining four protons (H-10, H-10′, H-11′ and H-11′) were in the 1.6–2.0 (3H) region and at 1.1 (1H) ^{13}C NMR: δ 117.5 (C-1), 143.9 (C-2), 136.0 (C-3), 131.7 (C-4), 126.4 (C-5), 148.5 (C-6), 15.6 (C-7), 33.3 (C-8), 19.3 (C-9), 39.9 (C-10), 23.9 (C-10), 23.9 (C-11), 123.5 (C-12), 137.0 (C-13), 41.8 (C-14), 26.2 (C-15), 168.7 and 20.6 (acetate). (Anal. calc. for $C_{17}H_{22}O_2$: C, 79.03; H, 8.58; O, 12.39%. Found: C, 79.04; H, 8.68; O, 12.23%.)

Curcuquinol monoisovalerate (**3**). Fraction D was rechromatographed over SiO_2, to give **3** (120 mg) as a pale yellow oil, $[\alpha]_D$ − 10° ($CHCl_3$; *c*, 1); UV $\lambda_{max}^{95\%EtOH}$ 209, 220 and 283 nm (ϵ, 14 000, 8000 and 2500); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 ($CDCl_3$, TMS int. standard) (hydroxyl) 1760 (ester) and 1630 (C–C double bonds); ^{1}H NMR (60 MHz): δ6.70 [*s*(br), 1H, H-6], 6.58 (*cs*, 1H, H-3), 5.88 (*br*, 1H, OH), 5.12 (*t* with further unresolved couplings, J_t = 7 Hz, 1H, H-12), 2.36 (*br*, 2H, –$COCH_2$), 2.08 (*s*, 3H, aromatic methyl), 1.68 and 1.55 (2*s*, *br*, 3H each, vinylic methyls) and 0.93 (*d*, J = 7 Hz, 9H, secondary methyl); ^{13}C NMR: δ 141.0 (C-1), 137.1 (C-2), 113.0 (C-3), 152.2 (C-4), 122.4 (C-5), 123.8 (C-6), 15.5 (C-7), 31.9 (C-8), 21.1 (C-9), 37.5 (C-10), 26.1 (C-11), 124.3 (C-12), 131.2 (C-13), 17.6 (C-14), 25.8 (C-15), 172.2, 43.3, 31.9, 22.5 and 22.5 ($COCH_2CHMe_2$); MS (70 eV) *m/z*: 318 $[M]^+$, 234 $[M - C_5H_8O]^+$, 218 $[M - C_5H_8O_2]^+$, 136 $[M - C_5H_8O_2$-4-methyl-1,3-pentadiene$]^+$ and 85 $[COCH_2CHMe_2]^+$.

The calculation for the assignment of the ring carbons of (**3**) were done from the experimental shifts [11] of thymol (**5**: R = iPr; HO at C-1) and isothymol (**6**: R = iPr; HO at C-4) by considering the effect [12] of an –OCOR group that shifts the *i*, *o*, *m* and *p* positions by +22.4, −7.1, +0.4 and −3.2, respectively. These calculations allowed the estimation of the chemical shifts of both possible thymoquinone monoesters (**7**: R=iPr; HO at C-1; ester–O at C-4 and **8**: R = iPr; HO at C-4; ester–O at C-1). Comparison of these shifts with those of the natural product are tabulated below:



	5	7	3	8	6
C-1	152.3	149.0	141.3	141.5	119.1
C-2	131.7	132.1	137.1	141.3	148.4
C-3	126.3	119.2	113.0(*d*)	113.9	113.5
C-4	121.9	144.3	152.2	150.2	153.4
C-5	136.6	129.5	122.4	122.0	121.6
C-6	116.3	116.7	123.8(*d*)	124.0	131.1

Good agreement of data was found between the experimental shifts of **3** and the estimated values for **8**, while there was no agreement between the experimental data of **3** and the calculated data of **7**.

Hydrolysis of curcuquinol monoisovalerate (**3**) A soln

containing 60 mg **3** in 2 ml MeOH was treated with 0.5 ml aq. 5% $KHCO_3$. The reaction mixture was refluxed for 2 hr, the MeOH evapd to a small vol. and the residue extracted with EtOAc. The aq. layer yielded, after treatment with aniline[14], 7 mg isovaleryl anilide, identified by direct comparison with an authentic sample.

The organic layer was evapd to dryness and chromatographed over SiO_2. This yielded 25 mg **4** as a yellow oil that showed: UV $\lambda_{max}^{95\%EtOH}$ 205, 253, 295 nm (ϵ, 7000, 10 500 and 700); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1655 (carbonyl) and 1615 (C–C double bonds); ^1H NMR (60 MHz, $CDCl_3$, TMS int. standard): δ 6.55 (*q*, $J = 1.8$, 1H, H-6), 6.47 (*d*, $J = 1.6$, 1H, H-3), 5.02 (*t* with further unresolved couplings, $J_t = 7$ Hz, 1H, H-12), 2.90 [multiplet (*br*), 1H, H-8], 2.00 (*d*, $J = 1.8$ Hz, 3H quinone methyl), 1.65 and 1.55 [2*s*(*br*), 3H, 3H each, vinylic methyls) and 1.1 (*d*, $J = 7$ Hz, 3H, secondary methyl). Direct comparison with a sample of curcuquinone (**4**) obtained by total synthesis [15], confirmed their identity.

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