Direct comparison (mmp, IR, NMR) with an authentic sample confirmed the identity. 3,5-Dinitroguaiacol showed marked antimicrobial activity against *B. subtilis.*

This is the first report of the isolation of dichloroacetamide and 3,5-dinitroguaiacol as natural products.

EXPERIMENTAL

Plant. Marginisporum aberrans (Yendo) Johansen et Chihara (Corallinaceae). The identification was carried out by Dr. T. Masaki, Faculty of Fisheries, Hokkaido University, Hakodate, Japan, *Source.* Cape Omaezaki, Shizuoka Prefecture, Japan.

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QUINONES FROM *PEREZIA RUNCINATA*

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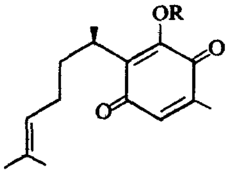
Key Word Index—*Perezia runcinata*; Compositae; roots; hydroxyperezone monoisovaleryl esters; isovalerylperezone; benzoquinones.

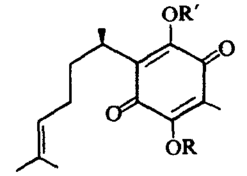
The hexane extract of 24 g of the roots of *Perezia runcinata* yielded 600 mg of a red oil which by PMR analysis showed the presence of perezone type signals, in addition to other peaks. The oil was separated by chromatography into perezone isovalerate (or *O*-isovalerylperezone) and the two hydroxyperezone monoisovalerates which were identified spectroscopically and by hydrolysis which gave the respective parent quinones and isovaleric acid.

Definitive proof of the structure of isovalerylperezone (**1b**) was obtained by alkaline hydrolysis which afforded perezone (**1a**) identified by standard procedures with an authentic sample [1] and isovaleric acid, characterized by its PMR spectrum and comparison of the corresponding anilide derivative with a sample prepared specifically [2].

Chemical confirmation of the hydroxyperezone esters was also obtained by alkaline treatment, which yielded hydroxyperezone and isovaleric acid, characterized as the anilide [2]. Thus only the position of the ester has to be defined in order to establish the complete structures.



1a: R = H
b: R = i-Val



2a: R = H, R′ = i-Val
2b: R = Me, R′ = i-Val
3a: R = i-Val, R′ = H
3b: R = i-Val, R′ = Me

In earlier work [3] we were able to define the esterifying position of a monoangeloyl hydroxyperezone by methylation of the free alcohol group, removal of the ester group and comparison of the product with a sample synthesized from perezone (**1a**). Therefore a portion of the red oil was esterified with Me_2SO_4, yielding a mixture of the two isomers **2b** and **3b** in almost equimolecular proportion, since in the PMR spectrum the hydroxyl signal originally at 7.04 ppm disappeared, two methoxyl singlets at 3.98 and at 4.02 ppm appeared and two quinonoid methyl signals at 1.88 and 1.93 ppm are now seen, instead of one signal at 1.91 ppm present before methylation.

The structural assignment of **2b** as the less polar constituent and of **3b** as the more polar one was done after detailed PMR considerations. Comparison of the chemical shifts of the quinonoid methyl group in 3-hydroxythymoquinone [4] with its corresponding methyl ether and in 6-hydroxythymoquinone also with the derived ether, reveals that the chemical shift difference for the *C*-methyl group associated with the methoxylation process is greater in the 3-substituted compounds than in the 6-substituted series, since in the first case the *O*-methyl group is introduced adjacent to the existing quinonoid methyl group. Similar differences are observed up on comparison of the spectrum of the mixture **2a** and **3a** with the spectra of the *O*-methylated derivatives **2b** and **3b**. Furthermore, treatment of this derivative with $Eu(DPM)_3$ shift reagent, revealed that greater shifts are induced to both the *C*-methyl and *O*-methyl groups of **3b** than to the same groups in **2b**. This is in agreement with the structural assignment of the isomers, since on one hand there appears to be no association at the ester carbonyls, as deduced from the chemical shifts of the methylene protons of the isovaleryl moiety and on the other hand, from the four available quinonoid carbonyls (two from each **2b** and **3b**) the less hindered one is that *alpha* to the methyl group in compound **3b** and therefore the shift reagent interaction occurs preferentially at that position.

EXPERIMENTAL

Mps are uncorrected. IR spectra in $CHCl_3$ and UV spectra in 95% EtOH; PMR spectra in $CDCl_3$ or CCl_4 with internal TMS; MS through the courtesy of Ing. F. Jáuregui (Laboratorio Central de la Secretaría de Hacienda y Crédito Público, México City).

Extraction of P. runcinata. Samples of *Perezia runcinata* Lag. ex D. Don were collected and classified by Professor J. Marroquín (Universidad Autónoma de Coahuila, to whom we are indebted) in March 1974 near Sabinas in the State of Nuevo León (México). The dried, ground roots (24 g) were extracted × 2 with 100 ml hexane under reflux for 8 hr. The combined extracts were evaporated to dryness and the residue (600 mg) was chromatographed over 50 g Si gel. The fractions eluted with pentane were combined yielding 170 mg of isovalerylperezone (**1c**) as a yellow oil, whose purity was tested by TLC using several solvents. It showed UV λ_{max} 204, 259 nm; ε, 25 200, 9600, IR bands at 1780 (enol ester carbonyl), 1670 (quinone carbonyls) and 1630 cm^{-1} (double bonds), PMR signals at 6.50 (*q*, $J = 1.6$ Hz, quinone proton), 5.01 (*m*, side chain vinylic proton), 2.84 (*m*, side chain methine), 2.41 (broad *s*, isovaleryl methylene), 2.01 (*d*, $J = 1.6$ Hz, quinone methyl), 1.66 and 1.53 (two broad *s*, vinylic methyls), 1.16 (*d*, $J = 7$ Hz, side chain secondary methyl) and 1.08 ppm (*d*, $J = 7$ Hz, isovaleryl methyls), MS peaks at *m/e* 332 (molecular ion), 248 (loss of isovaleryl), 192 (loss of isovaleryl and two CO), 166 (loss of isovaleryl and 4-methyl-1,3-pentadiene) and 85 (isovaleryl ion). The fractions eluted with pentane–C_6H_6 mixtures and with C_6H_6 were combined, yielding 320 mg of the mixture of the two monoisovalerylhydroxyperezones (**2a** and **3a**) as a red oil that showed a single spot on TLC, UV λ_{max} 201, 274 nm; ε 23 200, 13 200, IR bands at 3400 (hydroxyl), 1770 (enol ester carbonyl) and 1650 cm^{-1} (quinone carbonyls), PMR signals at 7.04 (hydroxyl, disappearing upon addition of D_2O), 5.00 (*m*, side chain vinylic proton), 2.97 (*m*, side chain methine), 2.41 (broad *s*, isovaleryl methylene), 1.91 (*s*, quinone methyl), 1.63 and 1.53 (two broad *s*, vinylic methyls), 1.19 (*d*, $J = 7$ Hz, side chain secondary methyl) and 1.08 ppm (*d*, $J = 7$ Hz, isovaleryl methyls), MS peaks at *m/e* 348 (molecular ion), 264 (loss of isovaleryl), 248 (loss of isovalerate) and 85 (isovaleryl ion).

Hydrolysis of isovalerylperezone (**1b**). A cold solution of 100 mg **1b** in 2 ml EtOH containing 2 ml 5% NaOH was stirred during 5 min. The violet mixture was acidified with cold dil. HCl and the resulting orange mixture extracted × 2 with Et_2O, dried and evaporated. Crystalization of the residue from Et_2O–pentane afforded perezone (**1a**) identified with an authentic specimen [5] by standard procedures. The mother liquors were treated with aniline, yielding 20 mg isovaleryl anilide, mp 106–108°, identified with a sample specifically prepared [2] for comparison.

Hydrolysis of monoisovalerylhydroxyperezones (**2a** *and* **3a**). 100 mg of the ester mixture was treated as described above, yielding 28 mg hydroxyperezone identified with an authentic sample [5] by standard procedures. The mother liquors revealed the presence of isovaleric acid characterized as its anilide.

Methylation of **2a** *and* **3a**. 200 mg of the ester mixture in 5 ml Me_2CO was stirred under reflux during 3 hr in the presence of 0.4 ml Me_2SO_4 and 800 mg anhydrous K_2CO_3. The reaction mixture was filtered and the ppt. washed with EtOAc. The combined soln was diluted with EtOAc and washed; the organic layer was dried and evaporated. The residue was chromatographed repeatedly over Si gel using pentane–hexane mixtures and monitoring by PMR. The less polar fractions yielded **2b** as a red oil that showed PMR signals at 5.0 (*m*, vinylic proton), 3.98 (*s*, methoxyl), 2.97 (*m*, side chain methine), 2.41 (broad *s*, isovaleryl methylene), 1.88 (*s*, quinone methyl), 1.67 and 1.53 (two broad *s*, vinylic methyls), 1.18 (*d*, $J = 7$ Hz, side chain secondary methyl) and 1.07 ppm (*d*, $J = 7$ Hz, isovaleryl methyls), MS molecular ion at *m/e* 362. The more polar fractions yielded **3b** as a red oil that showed the same PMR signals as **2b**, except the positions of the methoxyl and quinone methyl groups that are found now at 4.02 and at 1.93 ppm respectively. The molecular ion is also found at *m/e* 362.

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