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ABIETANE DITERPENOIDS FROM *SALVIA CHINOPEPLICA*

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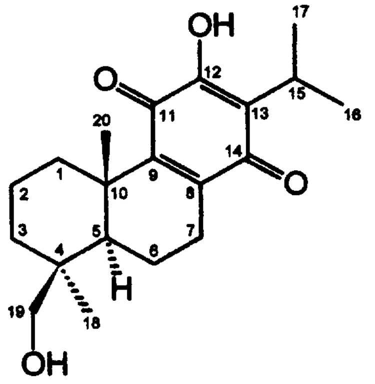
Key Word Index—*Salvia chinopeplica*; Labiatae; diterpenes; abietane; 19-hydroxy-royleanone; flavonoids.

Abstract—A new aromatic diterpene has been isolated from the leaves of *Salvia chinopeplica* and its structure established on the basis of spectral data as 19-hydroxy-royleanone. The known diterpenes carnosol, 16-hydroxy-carnosol, 16-hydroxy-20-deoxo-carnosol, 16-hydroxy-carnosic acid, 16-hydroxy-ferruginol, 7α-methoxy-rosmanol and 11,12,16-trihydroxy-20-nor-abietan-5(10),8,11,13-tetraen-l-one, as well as ursolic acid and the flavonoids velutin, salvigenin and 3,5-dihydroxy-7,4′-dimethoxy-flavone were also isolated from the same source. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Species of the genus *Salvia* have held a place of importance from ancient times, due to their widely reported uses as folk-medicinal plants [1], as well as to their commercial interest in the production of essential oils [2] and flavouring agents [3]. This group of plants has been characterized from a phytochemical standpoint by the production of flavonoids [4], monoterpenoids [5], triterpenoids [6] and mainly diterpenoids with an abietane or clerodane skeleton [7–10]. Many diterpenoids isolated from *Salvia* species have shown antioxidant [11], antifeedant [12], antibacterial [13] or cytotoxic [14] properties.

Continuing our studies on Latin American *Salvia* species, we have now examined the leaves of *S. chinopeplica* Epling, a mexican species endemic to Baja California. In this report, we describe the isolation and characterization of 19-hydroxy-royleanone (1), which is a new natural product. In addition seven known abietane diterpenoids, carnosol [15], 16-hydroxy-carnosol [16], 16-hydroxy-20-deoxo-carnosol [17], 16-hydroxy-carnosic acid [18], 16-hydroxy-ferruginol [19], 7α-methoxy-rosmanol [20] and 11,12,16-trihydroxy-20-nor-abietan-5(10), 8,11,13-tetraen-l-one [16], as well as ursolic acid and the flavonoids velutin [21], salvigenin [22] and 3,5-dihydroxy-7,4′-dimethoxy-flavone [23] have been identified.



(1)

RESULTS AND DISCUSSION

Acetone extraction of the air-dried leaves of *S. chinopeplica* afforded a crude extract, which was successively chromatographed on columns of silica gel and Sephadex LH-20. The fractions enriched in diterpenes were purified by preparative TLC, allowing the isolation of above named products. The identities of the known compounds were established by mixed mp, co-TLC, and by comparing their spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR and EI-MS) with those of authentic samples, except for the flavonoids which were identified by comparison of their physical constants and spectroscopic data with those reported in the literature [21–23].

Compound **1**, isolated as a yellow oil, had the molecular formula $C_{20}H_{28}O_4$, as deduced from high resolution EI mass spectrometry ([M]$^+$ at *m/z* 332.19946; requires 332.19876). The IR spectrum indicated the presence of hydroxyl groups (3380 cm^{-1})

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and a *p*-quinone moiety (1610, 1635 and 1670 cm^{-1}), which was correlated with the UV absorption maxima at 272 and 402 nm, indicating a 2-hydroxy-1,4-benzoquinone chromophore like that in royleanone [24]. The ^1H NMR spectrum of **1** did not show any signals in the olefinic proton region, but the ^{13}C NMR spectrum did show signals at δ_C 124.2, 140.9, 148.8 and 150.7 corresponding to four quaternary sp^2 carbons, as well as two signals at δ_C 183.0 and 187.8 assignable to quinone carbonyl carbons. The ^1H NMR spectrum of **1** also showed signals for two tertiary methyls (δ_H 1.04 and 1.26), an isopropyl group attached to a *p*-quinone ring (two 3H doublets, $J = 7.1$ Hz, at δ_H 1.19 and 1.22, 1H *sept.*, $J = 7.1$ Hz, at δ_H 3.15) and a hydroxymethylene group (AB system at δ_H 3.52 and 3.77, $J = 10.8$ Hz), which was also evidenced because in the EI mass spectrum a peak appeared at *m/z* 301 $[M-CH_2OH]^+$.

The above data suggested that **1** might be an abietane derivative with a ring C similar to that in royleanone [24, 25] and possessing one of the *gem*-dimethyl groups at C-4 oxidized to a primary hydroxyl group. In the ^{13}C NMR spectrum of **1** the signal corresponding to the hydroxymethylene carbon appeared at δ_C 65.7. Since it is known that in the ^{13}C NMR spectra of carbocyclic diterpenoids the signal due to a C-4 axial hydroxymethylene carbon appears upfield (*ca.* δ_C 63–66) to that corresponding to C-4 equatorial ones (*ca.* δ_C 70–73) [26], it is logical to assume a β-axial rather than an α-equatorial orientation for the hydroxymethylene group in compound **1**. This conclusion was also supported by the ^1H NMR spectrum, in which, the downward shifted position of the AB-doublets (δ_H 3.52 and 3.77) assigned to the hydroxymethylene protons, permitted an unambiguous establishment of the axial disposition of this group [27]. On the other hand it was evident that the deshielding effect of the axial hydroxymethylene group on the equatorial methyl group signal (H-18, δ_H 1.04) [28], caused a downfield shift relative to that in royleanone (δ_H 0.91) [25].

On the basis of the above spectral analysis, compound **1** was characterized as 19-hydroxy-royleanone, which is a new natural product. 19-Hydroxyl-abietane diterpenoids are relatively rare in Nature and even more so among the diterpenoids isolated from *Salvia* species. To the best of our knowledge, only one other diterpene of this type, 19-hydroxy-7α-acetoxyroyleanone, has been isolated from a *Salvia* species [29]. Finally, from a biogenetic point of view, it is interesting to note that the co-occurrence in *S. chinopeplica* of abietatriene phenolic and quinone diterpenoids, supports our previous conclusions [7] on the biosynthetic route proposed for these compounds.

EXPERIMENTAL

General. Mps: uncorr.; IR: film on NaCl or KBr pellets; UV: EtOH; ^1H and ^{13}C NMR: 200 and 25.15 MHz, respectively, $CDCl_3$ as solvent with TMS as int. standard; EI-MS and HREI-MS: direct inlet, 70 eV; Vacuum CC [30] was performed with silica gel 60 (70–230 mesh); prep. TLC: precoated Schleicher and Schüll (F-1500/LS 254).

Extraction and isolation of compounds. Dried and finely powdered leaves (360 g) of *Salvia chinopeplica* Epling, collected at Sierra de San Pedro Mártir, Baja California, México, in April 1993 (voucher specimen no. 8286 is on file in the BCMex Herbarium, Sciences Faculty of the Autonomous University of Baja California, Ensenada, México) were extracted with distilled Me_2CO at room temp. for 1 week. The remaining residue (44 g) after evapn of solvent under red. pres., was redissolved in Me_2CO and left at 4° overnight in a refrigerator. The white ppt. which formed was recovered by filtering, yielding 21 g of a crude material which was purified by repeated crystallization and then identified as ursolic acid (mmp, IR and ^1H NMR). The soln obtained was concd *in vacuo* to afford a dark brown residue (23 g), which was preabsorbed on silica gel and subjected to VCC over silica gel [30], using hexane with increasing amounts of EtOAc as eluent. Frs of 300 ml were collected and combined based upon TLC monitoring. The different frs were purified by chromatography on Sephadex LH-20 with 2:1:1 hexane–$CHCl_3$–MeOH as eluent and then by repeated flash chromatography or TLC. The compounds were obtained in the following sequence: 16-hydroxy-ferruginol (5 mg), carnosol (30 mg), 19-hydroxy-royleanone (**1**) (14 mg), velutin (25 mg), 7α-methoxy-rosmanol (8 mg), 11,12,16-trihydroxy-20-nor-abietan-5(10),8,11,13-tetraen-l-one (5 mg), 16-hydroxy-20-deoxo-carnosol (6 mg), 3,5-dihydroxy-7,4′-dimethoxy-flavone (6 mg), salvigenin (15 mg), 16-hydroxy-carnosol (118 mg) and 16-hydroxy-carnosic acid (45 mg).

19-Hydroxy-royleanone (**1**). Yellow oil. $[M]^+$ *m/z* 332.19946 (requires for $C_{20}H_{28}O_4$, 332.19876); UV λ_{max}^{EtOH} nm: 272, 402; IR ν_{max}^{film} cm^{-1}: 3380, 2940, 1670, 1635, 1610, 1460, 1255, 1030, 895, 790. ^1H NMR: δ 1.04 (3H, *s*, Me-18), 1.19, 1.22 (each 3H, *d*, $J = 7.1$ Hz, Me-16 and Me-17), 1.26 (3H, *s*, Me-20), 2.30 (2H, *m*, $W_{1/2} = 38.2$ Hz, H-7), 2.75 (2H, *m*, $W_{1/2} = 26.0$ Hz, 2 × H-1), 3.15 (1H, *sept*, $J = 7.1$ Hz, H-15), 3.52, 3.77 (each 1H, AB-system, $J = 10.8$ Hz, 2 × H-19), 7.23 (1H, *s*, —$\underline{O}\underline{H}$-12). ^{13}C NMR: δ 17.9 (*q*, C-17), 18.6 (*t*, C-6), 19.8 (*q*, C-16), 19.9 (*t*, C-2), 20.5 (*q*, C-18), 24.1 (*q*, C-20), 27.0 (*t*, C-7), 27.1 (*d*, C-15), 35.4 (*t*, C-3)*, 36.3 (*t*, C-1)*, 38.2 (*s*, C-10)†, 38.7 (*s*, C-4)†, 52.4 (*d*, C-5), 65.7 (*t*, C-19), 124.2 (*s*, C-13), 140.9 (*s*, C-8), 148.8 (*s*, C-9), 150.7 (*s*, C-12), 183.0 (*s*, C-11), 187.8 (*s*, C-14). EIMS, *m/z* (rel. int.): 332 $[M]^+$ (100), 317 $[M-Me]^+$ (4), 314 $[M-H_2O]^+$ (3), 302 (9), 301 $[M-CH_2OH]^+$ (13), 282 (7), 235 (13), 231 (16), 219 (23), 207 (13), 205 (13), 185 (18), 181 (35), 163 (22), 149 (16), 132 (41), 124 (54).

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