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TWO TERPENOIDS FROM *SALVIA BICOLOR*

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Abstract—Two new terpenoids, 20(*S*),24(*R*)-epoxy-dammar-12,25-diol-3-one and 12-methoxy-11,7-dihydroxy-dehydroabietane, were isolated from the roots of *Salvia bicolor*.

INTRODUCTION

The genus *Salvia* has been widely studied and has yielded a great number of products. The diterpenoids are usually of the abietane type as in *S. tomentosa* [1], or are quinones such as those found in *S. miltiorrhiza* [2–4], or have rearranged skeletons as in *S. aethiopis* [5]. Diterpenoids with a clerodane skeleton have been isolated from South American species, e.g. *S. melissodora* [6] and *S. splendens* [7] The most common triterpenoids are of the oleanene or ursene type [8–10], although other types have been isolated, e.g. lupanes from *S. phlomoides* [11] or friedelane from *S. glutinosa* [12]. We now report that *S. bicolor*, collected in Malaga, in the Andalusian region of Spain, contains two new substances: a diterpenoid and a dammarane-type triterpenoid product. This is the first time that a dammarane-type triterpenoid has been isolated from a *Salvia* species.

RESULTS AND DISCUSSION

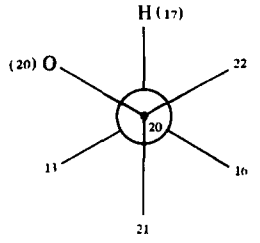
Compounds **1** and **2** were isolated from an acetone extract of the roots of *S. bicolor*. The IR spectrum of **1** showed absorptions due to hydroxyl and carbonyl groups ($\nu_{max}^{CHCl_3}$ 2970, 2890 and 1710 cm^{-1}). The ^1H NMR spectrum of **1** was very informative. It showed the presence of eight C-methyl singlets and two protons geminal to oxygen [δ3.85 (1H, *dd*) and 3.52 (1H, *ddd*)]. No signals due to olefinic or aldehyde protons were observed. Compound **1** was, therefore, probably a triterpenoid. Its ^{13}C NMR spectrum showed 30 signals: one carbonyl group (217.5 ppm), six methine carbon atoms [two (70.8 and 85.4 ppm) of them probably bearing an oxygen atom], nine methylenes, six tetrasubstituted carbon atoms [two (70 1 and 86.5 ppm) of them probably bearing an oxygen atom] and eight methyl groups. These data were sufficient to rule out the possibility of a pentacyclic triterpenoid of the β-amyrin type although this type of skeleton is very common in *Salvia* species [8–10]. It suggested other skeletal types such as lanostane or dammarane.

The mass spectrum of compound **1** showed its highest peak at *m/z* 459 $[M-15]^+$, compatible with a molecular formula of $C_{30}H_{50}O_4$. Another peak at *m/z* 415 $[M-59]^+$ and the base peak at *m/z* 143 were assigned to a side chain such as the one depicted in **1** [13]. The remaining data of the ^{13}C NMR spectrum were very much in agreement with the proposed structure (see Experimental) using appropriate models [14, 15]. The β-configuration assigned to the hydroxyl group at C-12 was suggested by the two large axial–axial couplings exhibited by the H-12 proton (1H, sextet, $J_{ax} = 10.4$, $J'_{ax} = 10.4$, $J_{eq} = 4.5$ Hz). As indicated in structure **1**, a 20(*S*),24(*R*)-configuration was assigned to these centres. It is known that dammarane derivatives with a 12β-hydroxyl group show a preferred conformation around the C-17(20) linkage which is fixed by strong hydrogen-bonding: O(12)-H---O(20) [14, 16]. This preferred conformation suggests that a change in the absolute configuration of C-20 should probably be reflected in the chemical shifts of the carbon atoms around this centre (see conformations **A** and **B** for C-20(*R*) and C-20(*S*), respectively).

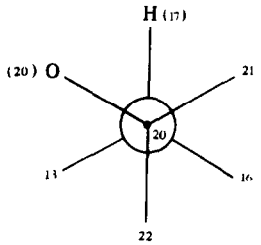
In fact, the chemical shifts of C-17, C-21, C-22, C-23 are almost identical in model compounds with C-20(*S*)-configuration [15] and triterpene **1**: C-17, 49.8; C-21, 26.1; C-22, 32.6 and C-23, 28.6 ppm.

Triterpenoid **1** afforded a dihydro derivative (**1a**) on treatment with sodium borohydride in methanol. The ^1H NMR spectrum of **1a** fully confirmed the location of the carbonyl group at C-3. A new signal at δ3.18 (1H, *q*, $J_{ax} = 11.0$, $J_{eq} = 5.2$ Hz) corresponded to a new proton geminal to a hydroxyl group. The two signals of the protons geminal to the hydroxyl groups present in the natural product did not change either their chemical shifts or their coupling patterns. The ^{13}C NMR spectrum of **1a** could be interpreted when allowance was made for the presence of a hydroxyl group at C-3 (β-configuration) and its effects on the carbon atoms of ring A. The remaining chemical shifts were essentially unaffected compared with those of compound **1** (see Experimental). This reduction product should correspond to pyxinol [17], and effectively its physical constants compared fairly well with those published for this compound. In addition, all the significant signals of the ^1H NMR spectrum of pyxinol [17] and those found for **1a** were identical. The ^{13}C NMR chemical shifts of **1a** closely corresponded to those published for the 3-monoacetyl derivative of pyxinol except for the A-ring carbon atoms, which showed the effects due to acetylation of the hydroxyl group at C-3. It has been shown that the absolute configuration at C-24 can be related to the chemical shifts of C-24, C-26 and C-27 [15]. Consequently, triterpenoid **1** was considered to be 20(*S*),24(*R*)-epoxy-dammar-12β,25-diol-3-one.

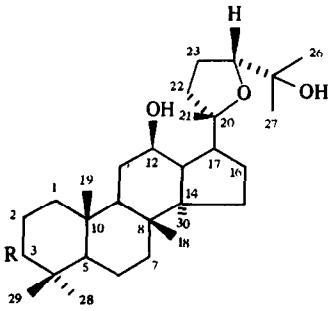
Compound **2** gave a mass spectrum with the highest peak at *m/z* 332. The ^{13}C NMR spectrum showed 21



A

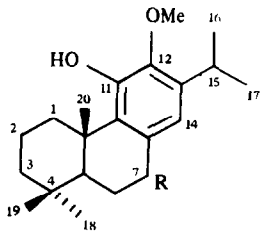


B



1 R=O

1a R = OH, H



2 R = H, OH

2a R = O

signals, the compound was probably methylated and the suggested molecular formula was $C_{21}H_{32}O_3$. There was no carbonyl absorption in the IR spectrum. The ^1H NMR spectrum gave much information. It contained an aromatic signal (1H, δ6.9), a singlet of three hydrogens of a methyl group which was linked to an oxygen atom at δ3.8 and a signal at δ6.1 which disappeared on treatment with deuterium oxide. There were also four C-methyl signals between δ1.2 and 0.95, one of them integrating for six hydrogen atoms. All these data pointed to an abietane-type compound with a phenolic hydroxyl and an aliphatic alcohol group. Comparison of the ^{13}C NMR data obtained for compound **2** with the data published for appropriate models [18, 19], in particular ferruginol acetate [18], allowed the location of the various substituents as indicated in structure **2**. In the ^{13}C NMR spectrum the estimated shifts caused by an axial hydroxyl group at C-7 explained the values assigned to C-5 (45.5, $\Delta\delta$ −4.6), C-6 (27.9, $\Delta\delta$8.8) and C-7 (69.2, $\Delta\delta$39.2). The remaining aliphatic carbons of rings A and B exhibited C-13 chemical shifts identical to those published for the model compound except for C-1 and C-20, which exhibited a shielding effect ($\Delta\delta$ −2.7 and −6.5) which was explained by the steric compression caused by a substituent at C-11 [20]. To confirm the position assigned to these substituents and more specifically the location of the methoxyl group, compound **2**, which was not methylated with diazomethane in ethyl ether, was subjected to Jones' oxidation to give a keto derivative (**2a**). The latter compound was identical to cryptojaponol [21] (direct comparison with an authentic sample).

EXPERIMENTAL

Mps (Kofler apparatus) are uncorr; IR: KBr, unless otherwise stated; MS: 70 eV, direct inlet; ^1H NMR and ^{13}C NMR: 90 and 22.5 MHz, respectively, in $CDCl_3$ soln with TMS as internal standard, unless otherwise stated. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. The plants of *S. bicolor* were collected in June 1982 in Algodonales (Malaga, Spain). Voucher specimens of the plants have been deposited at the Herbarium of the Faculty of Pharmacy (Universidad Complutense de Madrid).

Extraction and isolation of the components of S. bicolor. The aerial parts and roots of *S. bicolor* were processed separately Dried and finely powdered plant material (aerial parts 1 5 kg, roots 0.8 kg) was extracted with Me_2CO (5.0 and 3.0 l, respectively) at room temp. for 2 days. After filtration, the solvent was evapd, yielding a gum.

The crude extract of the aerial parts (29 7 g) was chromatographed on a short column [22] of silica gel 60 G. The only product isolated from this extract was ursolic acid, identified as the methyl ester of its acetylated derivative (mmp, IR, $[\alpha]_D$ and ^1H NMR). Similarly, the root extract (11.5 g) was chromatographed under suction on a short column of silica gel 60 G eluted with hexane–EtOAc (4:1). Fractions 10–13 contained two sub-

stances of similar R_f, which were separated by rechromatography using the same solvent system to give **1** and **2**. The final fractions of the first chromatography yielded a third component which was purified by rechromatography. This product decomposed on standing and could not be characterized further.

Triterpene **1** (20S,24R-*epoxy-dammar*-12β,25-*diol*-3-*one*). Thick oil; $[\alpha]_D$ 33.7° ($CHCl_3$; *c* 0 20); IR ν_{max}^{film} cm^{-1}: 3400, 1710, 750; MS *m/z* (rel. int.): 459 $[M-15]^+$ (25), 441 (20), 415 (60), 398 (100), 380 (25), 371 (50), 356 (60), 143 (100); ^{1}H NMR (300 MHz): δ3.85 (1H, *q*, J = 6.8, J' = 7.8 Hz, H.24), 3.525 (1H, *sextet*, J_{ax} = 10.4, J'_{ax} = 10.4, J_{eq} = 4.5 Hz, H-12) and 8 C-Me singlets at: 1.28, 1 27, 1.10 (H-26, H-27, H-21), 1.07 (H-19), 1.04 (H-29), 1.02 (H-18), 0.96 (H-28), 0.91 (H-30); ^{13}C NMR (75 MHz): δ217.5 (*s*, C-3), 86.5 (*s*, C-20), 85.5 (*d*, C-24), 70.8 (*d*, C-12), 70.1 (*s*, C-25), 55.3 (*d*, C-5), 52.0 (*s*, C-14), 49.8 (*d*, C-17), 49.5 (*d*, C-9), 47.9 (*d*, C-13), 47.3 (*s*, C-4), 39.8 (*t*, C-1), 39.6 (*s*, C-8), 36.8 (*s*, C-10), 34.1 (*t*, C-7), 34.0 (*t*, C-2), 32.6 (*t*, C-22), 31.7 (*t*, C-15), 31.2 (*t*, C-11), 28.6 (*t*, C-23), 27.9* (*q*, C-26), 27.6* (*q*, C-27), 26 6 (*q*, C-28), 26.1 (*q*, C-21), 25 0 (*t*, C-16), 20.9 (*q*, C-29), 19.6 (*t*, C-6), 18.0 (*q*, C-30), 16.1 (*q*, C-18), 15.1 (*q*, C-19).

1a (20(S),24(R)-*epoxy-dammar*-3β,12β,25-*triol*). Triterpenoid **1** was reduced with $NaBH_4$ in MeOH to yield **1a**, mp 220–221° (MeOH–H_2O). $[\alpha]_D$ 54.5° ($CHCl_3$; *c* 0.30); ^{1}H NMR (300 MHz): δ3 85 (1H, *dd*, H-24), 3 52 (1H, *ddd*, H-12), 3.18 (1H, *q*, J_{ax} = 11.0, J_{eq} = 5.2 Hz, H-3), 1.28 (3H, *s*, H-26), 1.27 (3H, *s*, H-27), 1.10 (3H, *s*, H-21), 0 98 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 0.90 (3H, *s*, H-30), 0.85 (3H, *s*, H-29), 0.77 (3H, *s*, H-28); ^{13}C NMR· δ86.5 (*s*, C-20), 85.5 (*d*, C-24), 78.9 (*d*, C-3), 71.1 (*d*, C-12), 70.1 (*s*, C-25), 56.1 (*d*, C-5), 52 1 (*s*, C-14), 50 6 (*d*, C-17), 49.5 (*d*, C-9), 48.1 (*d*, C-13), 39.8 (*s*, C-4), 39.1 (*s*, C-8), 39.1 (*t*, C-1), 37.2 (*s*, C-10), 34.9 (*t*, C-7), 32.6 (*t*, C-22), 31.4 (*t*, C-15), 31.3 (*t*, C-11), 29.7 (*q*, C-28), 28.6 (*t*, C-23), 28.0 (*t*, C-2), 27.9* (*q*, C-26), 27 5* (*q*, C-27), 26.1 (*q*, C-21), 25.0 (*t*, C-16), 18.3 (*t*, C-6), 18 2 (*q*, C-30), 16.3 (*q*, C-18), 15.5† (*q*, C-19), 15.3† (*q*, C-29).

Diterpene **2** (12-*methoxy*-7α,11-*dihydroxy-dehydroabietane*). Thick oil; $[\alpha]_D$ +20° ($CHCl_3$; *c* 0.30); IR ν_{max}^{film} cm^{-1}: 3500, 3380, 1720, 1650, 1610, 1570, 1465, 1420, 1390, 1375, 1365, 1330, 1310, 1245, 1215, 1030, 1015, 990 and 945; MS *m/z* (rel. int.): 332 $[M]^+$ (100), 317 (2), 315 (2), 314 (2), 299 (20), 289 (5), 267 (60), 257 (10), 229 (80), 225 (15), 208 (10), 187 (10); ^{1}H NMR: δ6.9 (1H, *s*, H-14), 6.1 (1H, *s*, phenolic OH), 4.7 (1H, *d*, J = 4 Hz, H-7), 3.8 (3H, *s*, OMe), 3.15 (1H, *m*, H-15) and C-Me singlets at 1.2 (6H), 1 1 (3H), 1.0 (3H), 0.95 (3H); ^{13}C NMR. δ144 1 (*s*, C-12), 138.7 (*s*, C-11), 134.4 (*s*, C-8), 134.4 (*s*, C-9), 133.1 (*s*, C-18) 119.1 (*d*, C-14), 69.2 (*d*, C-7), 61.7 (*q*, O-Me), 45.5 (*d*, C-5), 41 5 (*t*, C-3), 39.6 (*s*, C-10), 36.1 (*t*, C-1), 33.6 (*q*, C-18), 33.2 (*s*, C-4), 27.9 (*t*, C-6), 26.5 (*d*, C-15), 23 8* (*q*, C-16), 23 6* (*q*, C-17), 22.0 (*q*, C-19), 19.3 (*t*, C-2), 18.3 (*q*, C-20).

Compound **2a** (12-*methoxy*-11-*hydroxy-dehydroabitan*-7-*one*). Jones' oxidation of compound **2** (12 mg) yielded **2a** (8 mg), identical to cryptojaponol. ^{13}C NMR: δ199.2 (*s*, C-7), 149.1 (*s*, C-12), 146.5 (*s*, C-9), 139.0 (*s*, C-11), 129.7 (*s*, C-13), 128.8 (*s*, C-8), 117.3 (*d*, C-14), 61.9 (*q*, OMe), 50.3 (*d*, C-5), 41.2 (*t*, C-3), 40.2 (*s*, C-10), 36.2 (*t*, C-1), 35.6 (*t*, C-6), 33.5 (*s*, C-4), 33.1 (*q*, C-18), 26.3 (*d*, C-15), 23.6 (*q*, C-16)*, 23.5 (*q*, C-17)*, 21.5 (*q*, C-19), 19.0 (*t*, C-2), 18.0 (*q*, C-20).

*, † Interchangeable assignments.

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REFERENCES

1. Ulubelen, A. and Mıski, M. (1981) *J. Nat. Prod.* **44**, 119.
2. Nakao, M. and Fukushima, T. (1934) *J. Pharm. Soc Jpn.* **54**, 154.
3. Takiura, K. and Koizumi, K. (1962) *Chem Pharm. Bull* **10**, 112
4. Kakisawa, H., Hayashi, T. and Yamazaki, T (1969) *Tetrahedron Letters* 301.
5 Boya, M. T. and Valverde, S. (1981) *Phytochemistry* **20**, 1367.
6. Rodríguez-Hahn, L., Martínez Casas, G and Romo, J (1973) *Rev. Latinoam. Quím.* **4**, 93.
7. Savona, G., Paternostro, M. P., Piozzı, F. and Hanson, J. R. (1979) *J. Chem. Soc. Perkın Trans.* 1, 533.
8. Brieskorn, C. H. and Grossekettler, G (1964) *Arch. Pharm.* **297**, 456.
9 Ulubelen, A. and Brieskorn, C. H. (1975) *Phytochemistry* **14**, 1450.
10 González, A. G., Bretón, J L. and Fraga, B M (1971) *J. Chem. Soc. Chem. Commun.* 567
11. García Alvarez, M. C., Savona, G. and Rodríguez, B. (1981) *Phytochemıstry* **20**, 481.
12. Taylor, D. A. H. (1967) *J. Chem. Soc C* 490.
13. Nagai, M., Tanaka, N., Ichikawa, S. and Tanaka, O. (1968) *Tetrahedron Letters* 4239.
14. Asakawa, J., Kasaı, R., Yamasaki, K and Tanaka, O (1977) *Tetrahedron* **33**, 1935.
15. Tanaka, O. and Yahara, S. (1978) *Phytochemıstry* **17**, 1353.
16 Iljin, S. J , Malinovskaya, G. V., Uvakova, N. I. and Elyakov, G. B. (1982) *Tetrahedron Letters* **23**, 5067.
17 Yosioka, I., Yamauchi, H. and Kıtagawa, I. (1972) *Chem. Pharm. Bull (Tokyo)* **20**, 502.
18. Wehrli, F. W. and Nishida, T. (1979) *Prog. Chem. Org. Nat Prod* **36**, 1
19. Escudero, J., Pérez, L , Rabanal, R. M and Valverde, S (1983) *Phytochemıstry* **22**, 585.
20. Levy, C. and Nelson, G. L. (1972) *C-13 NMR for Organic Chemists*, p. 24. John Wiley, New York.
21. Matsumoto, T., Ohsuga, Y., Harada, S. and Fukuı, K. (1977) *Bull. Chem. Soc. Jpn.* **50**, 266.
22. Hunt, B. J. and Rigby, W. (1967) *Chem. Ind.* 1868