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DITERPENOIDS FROM *SALVIA GREGGII*

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Key Word Index—*Salvia greggii*; Labiatae; diterpenoids; isopimaric acid; 3β-hydroxy-isopimaric acid; 14α-hydroxy-isopimaric acid; 14α,18-dihydroxy-7,15-isopimaradiene; 7,8β-dihydrosalviacoccin.

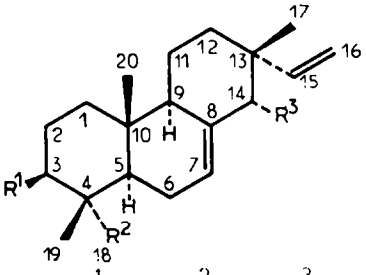
Abstract—From the aerial part of *Salvia greggii* a previously known diterpenoid, isopimaric acid, and four new natural substances, 3β- and 14α-hydroxy-isopimaric acids, 14α,18-dihydroxy-7,15-isopimaradiene and 7,8β-dihydrosalviacoccin, have been isolated. The structures of these diterpenoids have been established by chemical and spectroscopic means and, in some cases, by comparison with previously known synthetic compounds.

INTRODUCTION

In our search for new natural substances in the genus *Salvia* (Labiatae) [1–3], we have examined the aerial parts of *S. greggii*, a species originating from Mexico and Texas. From this plant we have isolated the previously known diterpenoid isopimaric acid (7,15-isopimaradien-18-oic acid, characterized as its methyl ester derivative **1**) [4–6], together with four new natural substances, three of which were the isopimarane derivatives 14α-hydroxy-7,15-isopimaradien-18-oic acid (**2**), 14α,18-dihydroxy-7,15-isopimaradiene (**3**) and 3β-hydroxy-7,15-isopimaradien-18-oic acid (characterized as its methyl ester derivative **4**), the other one being a neo-clerodane diterpenoid (**5**, 7,8β-dihydrosalviacoccin).

RESULTS AND DISCUSSION

The first of the new diterpenoids, compound **2**, had a molecular formula $C_{20}H_{30}O_3$ and its IR (see Experimental), ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively) showed characteristic features [7, 8] of a 7,15-isopimaradiene hydrocarbon skeleton possessing a C-18 carboxyl group (ν_{COOH} 3300–2500 br, 1700 cm^{-1}; $\delta_{C\text{-}18}$ 183.8 *s*, $\delta_{C\text{-}19}$ 17.1 *q*) and a secondary hydroxyl group (ν_{OH} 3500 cm^{-1}), the geminal proton of which appeared as a singlet at δ3.66 (Table 1) in the ^1H NMR spectrum. Thus, this secondary hydroxyl group must be placed at the C-14 position of a 7,15-isopimaradiene skeleton. In fact, the ^{13}C NMR spectrum of the diterpenoid (**2**, Table 2) was almost identical with that reported [9] for 14α-hydroxy-7,15-isopimaradien-18-oic acid methyl ester (**6**)—a substance prepared from sandaracopimaric acid [9, 10]—the only differences were the absence in the former of the signal of the methyl ester group and, consequently, the lower field resonance of the C-18 carbon atom (δ183.8 in **2** and δ179.0 in **6**). Moreover, ethereal diazomethane treatment of **2** yielded a substance whose ^1H NMR spectrum was identical with that of compound **6**. Thus, the new diterpenoid is 14α-hydroxy-7,15-isopimaradien-18-oic acid (**2**) or its enantiomer. Unfortunately, the physical data (mp, $[\alpha]_D$) of compound



	R^1	R^2	R^3
1	H	COOMe	H
2	H	COOH	OH
3	H	CH_2OH	OH
4	OH	COOMe	H
6	H	COOMe	OH

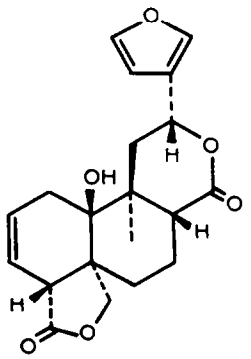


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Table 1. ^{1}H NMR data (90 MHz, $CDCl_3$ solution, δ values from TMS) of compounds **2**–**4** and **6**

	2	3	4	6
H-3α	*	*	4.04 *dd*	*
H-7	5.70 *m*	5.72 *m*	5.30 *m*	5.67 *m*
H-14β	3.66 *s*	3.60 *s*	*	3.61 *s*
H-15	5.90 †	5.93†	5.83 *dd*	5.92†
H_A-16	5.10 *dd*	5.08 *dd*	4.84 *dd*	5.09 *dd*
H_B-16	5.13 *dd*	5.11 *dd*	4.90 *dd*	5.12 *dd*
H_A-18	—	3.05 *d*	—	—
H_B-18	—	3.40 *d*	—	—
C–Me	1.27 *s*	0.90 *s*	1.26 *s*	1.22 *s*
	0.90 *s*	0.88 *s*	0.90 *s*	0.85 *s*
	0.89 *s*	0.87 *s*	0.86 *s*	0.83 *s*
COOMe	—	—	3.70 *s*	3.60 *s*
J(Hz)				
3α,2α	*	*	6	*
3α,2β	*	*	9.5	*
$W_{1/2}$ H-7	9	9	9	9
15,16A	17.6	18	17.4	18
15,16B	10.5	10.8	10.6	10.8
16A,16B	1.5	1.8	1.4	1.8
18A,18B	—	11	—	—

*Could not be identified.

†Six lines signal, the C part of an ABC system degenerated by the proximity of the C-14 hydroxyl group, see ref. [7].

Table 2 ^{13}C NMR chemical shifts (in δ values from TMS, $CDCl_3$ solution) of compounds **2** and **4**

C	2	4
1	38.7 *t**	37.4 *t*
2	18.0 *t*	26.6 *t*
3	36.8 *t*	75.6 *d*
4	46.2 *s*	53.0 *s*
5	44.5 *d*	45.8 *d*
6	25.1 *t*	24.9 *t*
7	127.2 *d*	120.7 *d*
8	136.9 *s*	135.6 *s*
9	46.9 *d*	52.2 *d*
10	34.7 *s*	35.0 *s*
11	19.2 *t*	20.1 *t*
12	27.3 *t*	36.0 *t*
13	41.0 *s*	36.8 *s*
14	79.3 *d*	46.3 *t*
15	146.3 *d*	150.2 *d*
16	113.8 *t*	109.4 *t*
17	22.0 *q*	21.5 *q*
18	183.8 *s*	178.0 *s*
19	17.1 *q*	11.2 *q*
20	15.1 *q*	15.3 *q*
OMe	—	51.8 *q*

*SFORD multiplicity.

6 were not available [9, 10; B. Delmond, personal communication] and the absolute configuration of the new diterpenoid cannot be established from the $[\alpha]_D$ values. However, on biogenetic grounds, we suppose that this diterpenoid belongs to the *normal* series, like isopimaric acid co-occurring in the same species.

Another of the new diterpenoids isolated from *S. greggii*, compound **3**, had a molecular formula $C_{20}H_{32}O_2$ and possessed a 7,15-isopimaradiene skeleton with a secondary hydroxyl group at the C-14 position as in compound **2** (one proton singlet signal at δ3.60, Table 1) and a C-18 hydroxymethylene grouping (AB system signals at δ3.05 and 3.40, J_{AB} = 11 Hz) instead of the C-18 carboxylic function of **2**. In agreement with this, reduction of hydroxy acid **2** with lithium aluminium hydride yielded a substance identical in all respects with the natural diol. Thus, this new diterpenoid is 14α,18-dihydroxy-7,15-isopimaradiene (**3**).

3β-Hydroxy-isopimaric acid was also present in the extract of *S. greggii*. It was purified after methylation with ethereal diazomethane and characterized as its methyl ester derivative **4**. Compound **4** showed characteristic features (Tables 1 and 2) of a 7,15-isopimaradiene derivative with an equatorial secondary hydroxyl group at the C-3 position (ν_{OH} 3460 cm^{-1}; geminal proton as a double doublet at δ4.04, $J_{aa'}$ = 9 Hz, $J_{ae'}$ = 6 Hz) and a carbomethoxyl group on C-18 (ν_{COOMe} 1725 and 1240 cm^{-1}). In particular, the ^{13}C NMR spectrum of **4** (Table 2) rigorously confirmed this structure with the carbon resonances being in agreement with a 7,15-isopimaradiene skeleton [8] possessing a carbomethoxyl group at the C-18 equatorial position (C-18, C-19 and OMe at δ178.0, 11.2 and 51.8, respectively) and a hydroxyl group equatorially attached to the C-3 position [C-3 at δ75.6, paramagnetic shifts on C-2 and C-4 (8.7 and 6.6 ppm, respectively) and diamagnetic shifts on C-1, C-10 and C-19 (−1.8, −0.5 and −6.3 ppm, respectively) with respect to methyl isopimarate (**1**)].

The last diterpenoid isolated from *S. greggii*, compound **5**, was a substance which showed identical physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^{1}H NMR and mass spectra) data with those reported [11] for 7,8β-dihydrosalviacoccin (**5**), a synthetic compound obtained by sodium borohydride treatment of salviacoccin [11].

To our knowledge, this is the first case in which compounds **2**–**5** have been isolated from a natural source.

EXPERIMENTAL

Mps are uncorr. For general details on methods see ref. [11]. Plant materials were collected in May 1984 in the Botanic Garden of Palermo, Italy, and voucher specimens are deposited in the Herbarium of this Centre.

Extraction and isolation of diterpenoids. Dried and finely powdered aerial parts of *S. greggii* A. Gray (200 g) were extracted with Me_2CO (2 l). at room temp. for 1 week. After filtration the solvent was evapd yielding a gum (10 g) which was subjected to dry-CC over silica gel (Merck No. 7734, deactivated with 15% H_2O, 300 g). Elution with petrol gave alkanes, fats and waxes which were rejected. Petrol–EtOAc (9:1) eluted isopimaric acid (200 mg), characterized as its Me ester derivative (**1**) [4–6]; petrol–EtOAc (4:1) eluted a mixture of ursolic and oleanolic acids, and petrol–EtOAc (2:1) successively eluted 14α-hydroxy-isopimaric acid (**2**, 180 mg), 14α,18-dihydroxy-7-15-isopimaradiene (**3**, 20 mg), 3β-hydroxy-isopimaric acid (purified as its Me ester derivative **4**, 50 mg) and 7,8β-dihydrosalviacoccin (**5**, 45 mg) [11].

The previously known diterpenoids, isopimaric acid Me ester (**1**) and 7,8β-dihydrosalviacoccin (**5**), were identified by their

physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^{1}H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

14α-*Hydroxy*-7,15-*isopimaradien*-18-*oic acid* (**2**). Mp 165–168° (Me_2CO–*n*-hexane); $[\alpha]_D^{19}$ −17.6° ($CHCl_3$; *c* 0.552); IR ν_{max}^{KBr} cm^{-1}: 3500, 3300–2500 (br), 2920, 2870, 1700, 1642, 1460, 1385, 1180, 1145, 1000, 930, 840, 720; ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 318 $[M]^+$ (23), 300 (48), 285 (30), 272 (18), 185 (24), 173 (52), 133 (64), 131 (58), 123 (88), 109 (69), 105 (94), 91 (87), 81 (86), 67 (72), 55 (100). (Found: C, 75.52; H, 9.37. $C_{20}H_{30}O_3$ requires: C, 75.43; H, 9.50%.)

14α,18-*Dihydroxy*-7,15-*isopimaradiene* (**3**). Mp 159–161° (EtOAc–*n*-hexane); $[\alpha]_D^{19}$ −36.7° ($CHCl_3$; *c* 0.115); IR ν_{max}^{KBr} cm^{-1}: 3340, 3280, 3080, 2930, 2870, 1640, 1460, 1390, 1065, 1055, 1005, 915, 830; ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 304 $[M]^+$ (16), 289 (5), 286 (17), 273 (15), 271 (12), 255 (24), 236 (16), 205 (13), 187 (18), 175 (17), 151 (20), 147 (24), 133 (22), 123 (82), 109 (100), 95 (38), 91 (36), 81 (43), 67 (31), 55 (47). (Found: C, 78.59; H, 10.47. $C_{20}H_{32}O_2$ requires: C, 78.89; H, 10.59%.)

3β-*Hydroxy*-7,15-*isopimaradien*-18-*oic acid methyl ester* (**4**). Mp 108–111° (from Me_2CO–*n*-hexane); $[\alpha]_D^{19}$ −37.8° ($CHCl_3$; *c* 0.397); IR ν_{max}^{KBr} cm^{-1}: 3460, 3300, 3090, 2960, 2860, 1725, 1640, 1435, 1385, 1240, 1145, 1055, 1025, 995, 910, 830; ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 332 $[M]^+$ (10), 314 (26), 299 (30), 272 (30), 239 (59), 201 (45), 187 (100), 145 (44), 131 (65), 119 (36), 105 (45), 91 (40), 81 (30), 67 (20), 55 (38). (Found: C, 75.81; H, 9.53. $C_{21}H_{32}O_3$ requires: C, 75.86; H, 9.70%.)

Diol **3** *from hydroxy acid* **2**. $LiAlH_4$ treatment of compound **2** (10 mg) in the usual manner yielded a substance (7 mg) identical in all respects (mp, mmp, IR, ^{1}H NMR, MS, TLC) with natural 14α,18-dihydroxy-7,15-isopimaradiene (**3**).

14α-*Hydroxy*-7,15-*isopimaradien*-18-*oic acid methyl ester* (**6**). Treatment of compound **2** (20 mg) with CH_2N_2–Et_2O yielded **6** (20 mg), mp 63–64° (MeOH); $[\alpha]_D^{20}$ −11.0° ($CHCl_3$; *c* 0.847); IR ν_{max}^{KBr} cm^{-1}: 3510, 3080, 3040, 2920, 2860, 1710, 1640, 1435, 1385, 1250, 1120, 1015, 905, 830; ^{1}H NMR (90 MHz, $CDCl_3$): see Table 1. (Found: C, 75.54; H, 9.59. Calc. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70%.)

7,8β-*Dihydrosalviacoccin* (**5**). Mp 268–270° (Me_2CO–Et_2O); $[\alpha]_D^{20}$ −123.8° (pyridine; *c* 0.112); mmp, TLC, IR, ^{1}H NMR and MS identical with those of 7,8β-dihydrosalviacoccin (mp 268–270°; $[\alpha]_D^{27}$ −127.5°) [11].

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REFERENCES

1. Hueso-Rodríguez, J. A., Jimeno, M. L., Rodríguez, B., Savona, G. and Bruno, M. (1983) *Phytochemistry* **22**, 2005.
2. Rodríguez, B., Pascual, C. and Savona, G. (1984) *Phytochemistry* **23**, 1193.
3. Rodríguez, B., Fernández-Gadea, F. and Savona, G. (1984) *Phytochemistry* **23**, 1805.
4. Antkowiak, W., ApSimon, J. W. and Edwards, O. E. (1962) *J. Org. Chem.* **27**, 1930 and 1931.
5. Ireland, R. E. and Newbould, J. (1963) *J. Org. Chem.* **28**, 23.
6. Weissmann, G. (1968) *Tetrahedron Letters* 2053.
7. Wenkert, E. and Beak, P. (1961) *J. Am. Chem. Soc.* **83**, 998.
8. Wenkert, E. and Buckwalter, B. L. (1972) *J. Am. Chem. Soc.* **94**, 4367.
9. Delmond, B., Taran, M., Valade, J., Petraud, M. and Barbe, B. (1981) *Org. Magn. Reson.* **17**, 207.
10. ApSimon, J. W. (1970) *J. Chem. Soc. Chem. Commun.* 83.
11. Savona, G., Bruno, M., Paternostro, M., Marco, J. L. and Rodríguez, B. (1982) *Phytochemistry* **21**, 2563.