Phytochemistry, Vol. 26, No. 12, pp. 3297–3299, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

DITERPENOID ABIETANE QUINONES ISOLATED FROM *SALVIA REGLA**

MIREYA HERNÁNDEZ, BALDOMERO ESQUIVEL, JORGE CÁRDENAS, LYDIA RODRÍGUEZ-HAHN† and T. P. RAMAMOORTHY‡

Instituto de Química; ‡Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F.

(*Received* 21 *January* 1987)

Key Word Index—*Salvia regla*; Labiatae, abietane quinones; diterpenoids; 19-hydroxy-7α-acetoxyroy-leanone; sessein; deacetylsessein.

Abstract—From the aerial parts of *Salvia regla*, a new abietane quinone diterpenoid has been isolated. Its structure, 19-hydroxy-7α-acetoxyroyleanone, was established by spectroscopic means. Oleanolic acid, β-sitosterol and sessein were also isolated. Deacetylsessein was found as a natural product in the same source.

INTRODUCTION

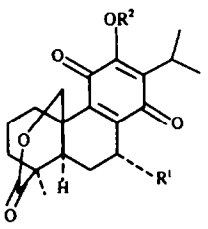
In continuation of our systematic phytochemical study of Mexican *salvia* spp. [1], we have analysed the aerial parts of *Salvia regla* Cav., *S. regla* has been classified [2] in the Section *Erytrostachys* (subgenus Calosphace). In addition to β-sitosterol and oleanolic acid, three abietane quinone diterpenoids were isolated. One of them was identified as sessein (**1**), previously isolated from *S. sessei* (M. Jiménez, E. Portugal, A. Lira, private communication), which also belongs to the Section *Erytrostachys* [2]. The other two diterpenoids, **2** and **5**, were new natural products related to sessein (**1**), and royleanone an abietane quinone commonly found in European and Asiatic *Salvia* spp [3, 4].

RESULTS AND DISCUSSION

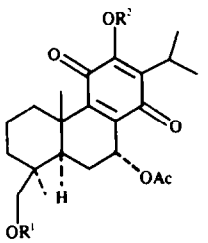
Deacetylsessein (**2**) had molecular formula $C_{20}H_{24}O_6$ and its IR spectrum showed the presence of a hydroxyl group (3577 cm^{-1}), a δ-lactone (1728 cm^{-1}), and a 2-hydroxy-1,4-benzoquinone group (3403, 1649, 1632 cm^{-1}). Its ^1H NMR spectrum (see Experimental) showed the presence of an isopropyl group attached to the benzoquinone moiety (δ 3.18, septet, J = 6 Hz, H-15, and two doublets at 1.23 and 1.22, Me-16 and Me-17). The C-20 methylene appeared as an AB system (δ4.21, 1H, *dd*, J = 9 and 1.5 Hz and 4.81, 1H, *d*, J = 9 Hz). A doublet of doublets observed at δ 4.76 (1H, J = 4 and 1.5 Hz) was assigned to the quasi equatorial H-7. Acetylation of **2** gave the diacetate derivative **3**, which was identical to the product obtained on acetylation of sessein (**1**).

The second new diterpenoid quinone was shown to be 19-hydroxy-7α-acetoxyroyleanone (**5**) on the basis of the following considerations. It showed molecular formula $C_{22}H_{30}O_6$ and its IR spectrum revealed the presence of a free hydroxyl group (3629 cm^{-1}), a 2-hydroxy-1,4-benzoquinone moiety (3391, 1642, 1609 cm^{-1}) and an ester carbonyl (1737 cm^{-1}). The ^1H NMR spectrum (see Experimental) was consistent with the structure (**5**) proposed for it, showing the isopropyl group attached to the quinone ring (δ3.16, 1H, septet, J = 6 Hz, H-15; 1.23 and 1.18, two doublets, J = 6 Hz, Me-16 and Me-17). A multiplet at 5.91 ($W_{1/2}$ = 6 Hz, 1H) was ascribed to H-7 by comparison with the ^1H NMR data described for 7α-acetoxyroyleanone [5]. Two singlets at 0.98 and 1.27 were assigned to Me-20 and Me-18 respectively. An AB system (3.52, 1H, and 3.73, 1H, J = 10 Hz) was due to the C-19 hydroxymethylene group. The β-axial orientation of the hydroxymethylene group was based [6] on the proton resonance data of **5** and its diacetate derivative **6**, and its biogenetic relationship with sessein (**1**). The ^{13}C NMR spectrum (Table 1) was in complete agreement with the structure proposed. The assignments were made by comparison with the data of similar structures [7, 8].

The new diterpenoid quinone 19-hydroxy-7α-acetoxy-royleanone (**5**) is therefore an isomer of the 20-hydroxy-7α-acetoxyroyleanone isolated from *S. lanata* [9].



	R^1	R^2
1	OAc	H
2	OH	H
3	OAc	Ac
4	H	H



	R^1	R^2
5	H	H
6	Ac	Ac

*Contribution No. 862 of the Instituto de Química, UNAM.
†Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR chemical shifts of compound 5 (20 MHz, $CDCl_3$, TMS as int. standard)

C	δ	C	δ
1	36.14 *t*[a]	11	183.82 *s*
2	19.75 *t*	12	151.04 *s*
3	35.58 *t*[a]	13	124.94 *s*
4	39.01 *s*[b]	14	185.44 *s*
5	46.72 *d*	15	26.96 *d*
6	25.29 *t*	16	18.86 *q*[c]
7	64.68 *d*	17	18.68 *q*[c]
8	149.88 *s*	18	21.03 *q*
9	139.42 *s*	19	65.94 *t*
10	38.28 *s*[b]	20	24.30 *q*
		-OCOMe	169.44 *s*
		-OCOMe	19.93 *q*

SFORD multiplicites are in parenthesis.
[a-c] Values in any vertical column can be interchanged.

EXPERIMENTAL

Mps are uncorr. MS were obtained by direct inlet at 70 eV. ^{1}H NMR and ^{13}C NMR spectra were performed at 80 and 20 MHz respectively, in $CDCl_3$ soln with TMS as int. standard. Plant material was collected in September 1985, at Amealco, Querétaro (México) and a voucher specimen (MEXU 252485) was deposited at the Herbarium of the Instituto de Biologia, UNAM.

Isolation of chemical constituents of S. regla. Dried and powdered aerial parts of *S. regla* (1800 g) were extracted with Me_2CO at room temp. for 8 days. Evaporation of the solvent yielded a gum (120 g) which was subjected to dry CC over silica gel (1000 g) deactivated with 10% H_2O, using as eluents petrol–EtOAc mixtures of increasing polarity. Elution with petrol–EtOAc (19:1) gave β-sitosterol, mp 130–134° (Me_2CO–MeOH), identified by comparison with an authentic sample.

Elution with petrol–EtOAc (9:1) left 2.14 g of a triterpenoid acid which was identified as oleanolic acid by comparison of its methyl ester with an authentic sample (mmp, IR and ^{1}H NMR spectra).

The fractions eluted with petrol–EtOAc (4:1) (8.9 g) were rechromatographed over 450 g of silica gel deactivated with 5% H_2O using a mixture of Me_2CO–CH_2Cl_2 (3:97) as eluent. From the first fractions of this chromatography, 6 g (0.33% dry wt) of 1 were isolated by crystallization with Me_2CO–*i*-propyl ether: mp 187–189°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3411, 1747, 1731, 1645, 1620, 1401, 1371; ^{1}H NMR ($CDCl_3$): δ 7.0 (1H, *s*, -OH at C-12), 5.96 (1H, *dd*, *J* = 4 and 1.5 Hz, H-7), 4.21 (1H, *dd*, *J* = 9 and 1.5 Hz H-20 *pro-S*), 4.81 (1H, *d*, *J* = 9 Hz, H-20 *pro-R*), 3.15 (1H, *sept*, *J* = 6 Hz, H-15), 2.85 (1H, *br d*, *J* = 12 Hz, H-1β), 2.05 (3H, *s*, -OCOMe at C-7), 1.22 (3H, *s*, Me-18), 1.23 and 1.18 (2*d*, 3H each, *J* = 6 Hz, Me-16 and Me-17); MS *m/z* (rel. int.): 402 [M]$^+$ (6), 360 (18), 343 (21), 342 (100), 314 (20), 298 (21), 285 (30), 284 (98), 269 (25), 256 (19), 241 (19), 213 (12), 128 (13), 91 (21), 83 (20), 77 (16), 69 (15), 55 (16), 43 (41). Compound 1 was previously isolated from *S. sessei*.

19-Hydroxy-7α-acetoxyroyleanone (5) was isolated from the mother liquors of 1, 0.115 g (0.0064% dry wt) by prep. TLC using a mixture of petrol–EtOAc (7:3) as eluent system. Mp 170–173°;

$$[\alpha]^{20} = \frac{0 \quad -125 \quad -161 \quad -163 \quad -161}{583 \quad 578 \quad 546 \quad 436 \quad 365}$$

(MeOH *c* 0.2); UV λ_{max}^{MeOH} nm (ε): 204 (14297), 272 (11087), 406 (260); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3629, 3391, 1737, 1642, 1609, 1397, 1374; ^{1}H NMR ($CDCl_3$): 7.12 (1H, *s*, exchangeable with D_2O, -OH at C-12), 5.91 (1H, *m*, $W_{1/2}$ = 6 Hz, H-7), 3.52 (1H, *d*, *J* = 10 Hz, H-19), 3.73 (1H, *d*, *J* = 10 Hz, H-19), 3.16 (1H, *sept*, *J* = 6 Hz, H-15), 2.75 (1H, *br d*, *J* = 12 Hz, H-1β), 2.05 (3H, *s*, -OCOMe), 1.27 (3H, *s*, Me-18), 1.23 and 1.18 (2*d*, 3H each, *J* = 6 Hz, Me-16 and Me-17), 0.98 (3H, *s*, Me-20); ^{13}C NMR ($CDCl_3$) see Table 1; MS *m/z* (rel. int.): 390 [M]$^+$ (5), 349 (12), 348 (55), 333 (12), 330 (35), 300 (19), 245 (12), 231 (14), 131 (12), 105 (12), 91 (19), 83 (18), 79 (11), 77 (12), 69 (15), 55 (25), 45 (12), 43 (100), 41 (30), ($C_{22}H_{30}O_6$ requires M$^+$ at *m/z* 390).

The following fractions gave 2 (0.292 g, 0.016% dry wt), mp 105–107° (Me_2CO–*i*-propyl ether), UV λ_{max}^{MeOH} nm (ε): 204 (8720), 270 (4972); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3577, 3403, 1728, 1649, 1632, 1611, 1380; ^{1}H NMR ($CDCl_3$): δ 7.1 (1H, *s*, -OH at C-12), 4.76 (1H, *dd*, *J* = 4 and 1.5 Hz, H-17), 4.21 (1H, *dd*, *J* = 9 and 1.5 Hz, H-20 *pro-S*), 4.81 (1H, *d*, *J* = 9 Hz, H-2; *pro-R*), 3.18 (1H, *sept*, *J* = 6 Hz, H-15), 2.85 (1H, *br d*, *J* = 12 Hz, H-1β), 2.25 (1H, exchangeable with D_2O, -OH at C-7), 1.33 (3H, *s*, Me-18), 1.23 and 1.22 (2*d*, 3H each, *J* = 6 Hz Me-16 and Me-17); MS *m/z* (rel. int.): 360 [M]$^+$ (17), 343 (14), 342 (100), 332 (22), 330 (42), 314 (22), 302 (32), 299 (10), 298 (11), 296 (12), 287 (45), 284 (23), 269 (11), 259 (18), 256 (12), 241 (14), 213 (12), 190 (15), 115 (17), 91 (19), 83 (20), 43 (22), ($C_{20}H_{24}O_6$ requires M$^+$ at *m/z* 360).

Acetylation of 1. Compound 1 (40 mg), in C_5H_5N (0.5 ml), was treated with 2 ml of Ac_2O at room temp. for 15 min. After usual work-up, the crystalline product 3 (42 mg) was obtained, mp 176–179°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1732, 1661, 1618, 1407, 1370; ^{1}H NMR ($CDCl_3$): 5.96 (1H, *dd*, *J* = 6 and 1.5 Hz, H-7), 4.23 (1H, *dd*, *J* = 9 and 1.5 Hz, H-20 *pro*-S), 4.87 (1H, *d*, *J* = 9 Hz, H-20 *pro*-R), 3.11 (1H, *sept*, *J* = 6 Hz, H-15), 2.66 (1H, *br d*, *J* = 12 Hz, H-1β), 2.35 (3H, *s*, -OCOMe at C-12), 2.06 (3H, *s*, -OCOMe at C-7), 1.23 (3H, *s*, Me-19), 1.20 and 1.18 (2*d*, 3H each, *J* = 6 Hz, Me-16 and Me-17); MS *m/z* (rel. int.): 444 [M]$^+$ (0.2), 443 (13), 342 (53), 326 (11), 285 (14), 284 (38), 55 (10), 43 (100), ($C_{24}H_{28}O_8$ requires M$^+$ at *m/z* 444).

Hydrogenolysis of 1. Compound 1 (50 mg), in EtOAc (5 ml) was hydrogenolysed using Pd-C (5%, 12 mg) as catalyst, for 30 min. After usual work-up 42.7 mg of 4 were obtained as a crystalline product; mp 169–172°; UV λ_{max}^{MeOH} nm (ε): 208 (11548), 276 (8175), 304 (2272), 402 (151); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3397, 1725, 1639, 1610, 1400, 1381; ^{1}H NMR ($CDCl_3$): 7.05 (1H, *s*, exchangeable with D_2O, -OH at C-12), 4.31 (1H, *dd*, *J* = 9 and 1.5 Hz, H-20 *pro-S*), 4.83 (1H, *d*, *J* = 9 Hz, H-20 *pro-R*), 3.15 (1H, *sept*, *J* = 6 Hz, H-15), 1.20–1.28 (9H, overlapped signals for Me-16, 17 and 18); MS *m/z* (rel. int.): 344 [M]$^+$ (100), 287 (15), 286 (65), 271 (28), 230 (16), 91 (16), 83 (11), 55 (13), 43 (15), 41 (15), ($C_{20}H_{24}O_5$ requires M$^+$ at *m/z* 344).

Acetylation of 2. Compound 2 (34 mg) in C_5H_5N (0.5 ml) was treated under the same conditions as those described for 1. After usual work up 52 mg of crude product were obtained. After purification by flash chromatography (petrol–EtOAc, 9:1), compound 3 was obtained.

19-Acetoxy-7α-acetoxyroyleanone (6). Product 5 (50 mg) in C_5H_5N (0.5 ml) was acetylated under the same conditions described for 1. The crude product of the reaction was purified by flash chromatography (petrol–EtOAc, 9:1) to give compound 6 (35 mg), mp 155–158° UV λ_{max}^{MeOH} nm (ε): 204 (9177), 261 (15664), 400 (316); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1771, 1737, 1666, 1611, 1372; ^{1}H NMR ($CDCl_3$): 5.92 (1H, *m*, $W_{1/2}$ = 6 Hz, H-7), 3.95 (1H, *d*, *J* = 9 Hz, H-19), 4.18 (1H, *d*, *J* = 9 Hz, H-19), 3.10 (1H, *sept*, *J* = 6 Hz, H-15), 2.65 (1H, *br d*, *J* = 10 Hz, H-1β), 2.35 (3H, *s*, -OCOMe at C-12), 2.06 (3H, *s*, -OCOMe at C-19), 2.05 (3H, *s*, -OCOMe at C-7), 1.28 (3H, *s*, Me-18), 1.23 and 1.16 (2*d*, 3H each, *J* = 6 Hz, Me-16 and Me-17), 0.97 (3H, *s*, Me-20); MS *m/z* (rel. int.): 474 [M]$^+$ (1.3), 432 (21), 432 (21), 414 (20), 399 (10), 391 (12),

390 (45), 373 (20), 372 (100), 357 (10), 330 (28), 313 (18), 312 (63), 299 (18), 297 (28), 283 (13), 269 (18), 244 (16), 83 (13), 43 (98), ($C_{26}H_{34}O_8$ requires M^+ at m/z 474).

Acknowledgements—We wish to thank Messrs R. Villena, H. Bojórquez, L. Velasco and R. Gaviño for spectroscopic assistance. The authors are also indebted to Mrs Elizabeth Argüelles for helping us in the plant material collection. This work was supported in part by CONACyT, México, project PCCBBNA 021142.

REFERENCES

1. Esquivel, B., Cárdenas, J., Ramamoorthy, T. P. and Rodríguez-Hahn, L. (1986) *Phytochemistry* **25**, 2381.
2. Epling, C. (1939) *Rep. Spec. Nov. Beih.* **110**, 1.
3. Hueso-Rodríguez, J. A., Jimeno, M. L., Rodríguez, B., Savona, G. and Bruno, M. (1983) *Phytochemistry* **22**, 2205.
4. Patudin, A., Romanowa, A., Sokolow, W. S. and Pribylowa, G. (1974) *Planta Med.* **26**, 201.
5. Yoshizaki, F., Rüedi, P. and Eugster, C. H. (1979) *Helv. Chim. Acta* **62**, 2754.
6. Gaudemer, A., Polonsky, J. and Wenkert, E. (1964) *Bull. Soc. Chim. France* 407.
7. Wehrli, F. W. and Nishida, T. (1979) *Prog. Chem. Nat. Prod.* **36**, 1.
8. Joseph-Nathan, P., Abramo-Bruno, D. and Ortega, D. A. (1981) *Org. Magn. Res.* **15**, 311.
9. Mukherjee, K. S., Ghosh, P. K. and Mukherjee, R. K. (1983) *Phytochemistry* **22**, 1296.