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ABIETANE AND CLERODANE DITERPENES FROM *SALVIA REGLA**

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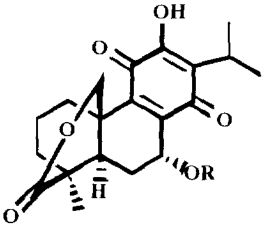
Key Word Index—*Salvia regla*; Labiatae; diterpenes; *neo*-clerodanes; abietanes.

Abstract—The known ursolic and oleanolic acids, the abietane quinone diterpenes, sessein, and deacetylsessein, together with the also known clerodane diterpenes hardwickiic and clerodermic acids were isolated from the aerial parts of *Salvia regla*. The structure of a novel compound, reglin, present in this species was established by spectral means as deacetyloxysessein-7α-(3β-hydroxy-olean-12-en-28-oate).

INTRODUCTION

A previous paper [1] describes the isolation of β-sitosterol, oleanolic acid, sessein (**1**), deacetylsessein (**2**) and 19-hydroxy-7α-acetoxyroyleanone from the aerial parts of *Salvia regla* Cav. (Sect. *Erytrostachys*, subgenus Calosphace) collected in Querétaro, México. We have now analysed a population of this species collected in Oaxaca, México. In addition to a complex mixture of triterpenes, consisting mainly of ursolic and oleanolic acids, we have isolated and identified the abietane quinone diterpenes **1** and **2** [1, 2], together with the *neo*-clerodane diterpenes, hardwickiic (**3**) [3, 4] and clerodermic acids (**4**) [5], and the new compound reglin (**5**) whose structure elucidation is described below.



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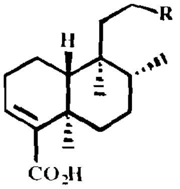

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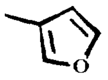

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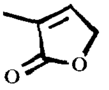

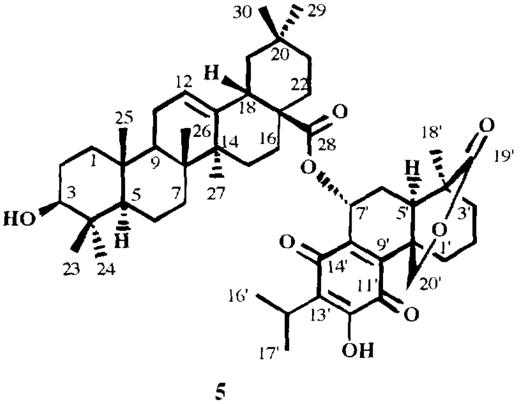

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RESULTS AND DISCUSSION

The FAB-mass spectrum of reglin (**5**) displayed a prominent $[MH]^+$ at m/z 799 which was accompanied by a peak at m/z 781, representing loss of H_2O. High resolution FAB-mass spectral analysis indicated that **5** had the molecular formula $C_{50}H_{70}O_8$. It exhibited IR absorptions for hydroxyl (3611, 3411 cm^{-1}), ester, δ-lactone (1730 cm^{-1}) and 2-hydroxy-1,4-benzoquinone (1647, 1616 cm^{-1}) groups. In its ^1H NMR spectrum (see Experimental) two sets of signals were observed. The first one was due to an abietane quinone diterpene and it was almost superimposable on the spectrum of sessein (**1**), although in **5** the OH-7′ group was not esterified by acetic acid, but by a different acid function. The triterpenoid nature of this acid was suspected from the second set of signals in which seven 3H singlet signals due to tertiary methyl groups, one signal at δ3.2, attributed to a carbinolic proton, and one broad triplet at δ5.28, assigned to a vinylic proton, were observed. These spectral data strongly supported the structure of a 3β-hydroxyolean-12-en-28-oate for the triterpenoid acyl moiety in **5**. Additional evidence for this conclusion was obtained from the similarity of these data with those reported for methyloleanolate [6].

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The major support for structure **5** was provided by ^{13}C and DEPT NMR experiments, which led to the ^{13}C assignments shown in Table 1. These were compared with those previously reported for methyl-oleanolate [7, 8] and sessein [9] and were found to be almost identical.

The FAB collisionally induced dissociation tandem mass spectrometry (FAB-CID-MS/MS) of $[\mathrm{MH}]^+$ at *m/z* 799 provided confirmatory evidence for the proposed structure. Prominent peaks representing loss of a sessein moiety from cleavages around the ester linkage were detected at *m/z* 439 $\mathrm{MH} - \mathrm{C_{20}H_{24}\,O_6}]^+$ and 411 $[\mathrm{MH} - \mathrm{C_{21}H_{24}O_7}]^+$.

Due to the coexistence of oleanolic acid and deacetylsessein in the plant, there was the possibility that reglin could be an artifact formed during the isolation process. This possibility was discounted by the demonstration than when an ethyl acetate solution of the mixture of ursolic and oleanolic acids and deacetylsessein was stirred with bentonite (an acidic material containing *ca* 70% silica gel) for 48 hr no reglin was obtained.

Recently, we have reported on the presence of abietanes in the roots and clerodanes in the aerial parts of *Salvia lavanduloides* [10], but *Salvia regla* is, to our knowledge, the first *Salvia* species in which both types of diterpenes have been found in the aerial parts.

EXPERIMENTAL

Isolation of compounds **1–5**. Ground and dried aerial parts of *Salvia regla* Cav. (890 g) collected near Tamazulapan, Oaxaca State, México (voucher specimen deposited at the National Herbarium, MEXU-592188) were extracted with $\mathrm{Me_2CO}$ and MeOH to obtain, after solvent evapn 100.1 and 46.7 g of extracts, respectively. An $\mathrm{Me_2CO}$ soluble fr. of the MeOH extract was obtained (31 g), combined with the $\mathrm{Me_2CO}$ extract and fractioned by CC over silica gel using mixtures of petrol–EtOAc of increasing polarities as eluent. Frs eluted with petrol and petrol–EtOAc (19:1) contained **3**, which was purified by partition between petrol–3% NaOH. The aq. phase was treated with 5% HCl aq. and extracted with petrol. The organic phase was washed with $\mathrm{H_2O}$ and dried over $\mathrm{Na_2SO_4}$. The residue was purified by CC (silica gel, petrol) and crytallized from petrol to obtain 11.82 g of **3**, mp 118–120 (lit. [3]: 106–107). Hardwickiic acid (**3**) was identified by comparision of its spectral data (IR, ^{1}H and ^{13}C NMR) and those of its methyl derivative with those reported in the lit. [3, 10].

Table 1. ^{13}CNMR spectral data of compound **5** (75 MHz, $\mathrm{CDCl_3}$)

C	δ	C	δ
1	38.5 *t*	26	17.4 *q*
2	27.2 *t*	27	25.6 *q*
3	79.1 *d*	28	175.4 *s*
4	38.8 *s*	29	33.0 *q*
5	55.3 *d*	30	23.6 *q*
6	18.4 *t*	1′	35.6 *t*
7	32.8 *t*	2′	20.9 *t*
8	39.5 *s*	3′	40.4 *t*
9	47.7 *d*	4′	41.9 *s*
10	37.1 *s*	5′	42.9 *d*
11	23.3 *t*	6′	25.5 *t*
12	122.3 *d*	7′	61.5 *d*
13	143.4 *s*	8′	140.7 *s*
14	41.9 *s*	9′	143.8 *s*
15	27.3 *t*	10′	37.6 *s*
16	23.5 *t*	11′	183.4 *s*
17	46.8 *s*	12′	150.6 *s*
18	41.3 *d*	13′	125.9 *s*
19	46.1 *t*	14′	184.2 *s*
20	30.6 *s*	15′	24.6 *d*
21	34.0 *t*	16	20.1 *q*
22	32.4 *t*	17′	19.5 *q*
23	28.1 *q*	18′	23.1 *q*
24	15.6 *q*	19′	174.7 *s*
25	15.4 *q*	20′	73.4 *t*

Triterpenoid acids were present in frs eluted with petrol–EtOAc (4:1, 7:3, 3:2 and 1:1) together with other compounds. These frs were combined and partitioned between petrol–$\mathrm{C_6H_6}$ (3:2) and MeOH–$\mathrm{H_2O}$ (4:1). The non-polar phase containing mainly triterpenoid acids was decolorized with activated C and submitted to CC (silica gel, petrol–EtOAc, 9:1) to obtain, after crystallization, 5.12 g of a mixture of triterpenoid acids. This mixture (238 mg) was methylated with $\mathrm{CH_2N_2}$ as usual to obtain, after CC, 163 mg of a mixture of ursolic and oleanolic methyl esters. The polar phase gave, after successive CC (silica gel, petrol–EtOAc gradient elution) and crystallization, 12.5 g (1.44% dry wt) of sessein (**1**), mp 208–210° (lit.: 187–189° [1], 208–210° [2]); 151 mg (0.017% dry wt) of deacetylsessein (**2**), mp 151–153° (lit.: 105–107° [1], 114–116° [2]), which was identified by comparison with an authentic sample (TLC, IR, ^{1}H NMR); 2.35 g (0.264% dry wt) of clerodermic acid (**4**), mp 166–168° (lit.: 161–162° [5]) and 58.1 mg of reglin (**5**), mp 227–233°, $[\alpha]_D + 64.4$; IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3611, 3411, 1730, 1646, 1616, 1465, 1393, 1364, 1145, 1030, 909; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ 3.20 (1H, superimposed, H-3), 5.28 (1H, *br t*, $J = 4$ Hz, H-12), 2.83 (1H, superimposed H-18_β), 0.98 (3H, *s*, H-23), 0.85 (3H, *s*, H-24), 0.92 (3H, *s*, H-25), 0.78 (3H, *s*, H-26), 1.11 (3H, *s*, H-27), 0.87 (3H, *s*, H-29), 0.95 (3H, *s*, H-30), 2.83 (1H, superimposed *br d*, $J = 12$ Hz, H-$1'_\beta$), 5.96 (1H, *dd*, $J = 12$, 4 Hz, H-$7'_\beta$), 3.17 (1H, *sept*, $J = 7$ Hz, H-15′), 1.20 (3H, *d*, $J = 7$ Hz, H-16′), 1.18 (3H, *d*, $J = 7$ Hz, H-17′), 1.18 (3H, *s*, H-18′), 4.84 (1H, *d*, $J = 12$ Hz, H-$20'_{pro\ \mathrm{R}}$), 4.23 (1H, *dd*, $J = 12$, < 1 Hz, H-$20'_{pro\ \mathrm{S}}$), 6.97 (1H, *s*, OH-12′).

Treatment of deacetylsessein (**2**) with bentonite. Bentonite (2 g) was added to a soln of the mixture of ursolic and oleanolic acids (73 mg) and deacetylsessein (**2**) (23.2 mg) in EtOAc (15 ml). The suspension was stirred at room temp. for 48 hr, and monitored for chemical reactions by TLC. The suspension was then filtered off and the starting materials recovered.

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REFERENCES

1. Hernández, M., Esquivel, B., Cárdenas, J., Rodríguez-Hahn, L. and Ramamoorthy, T. P. (1987) *Phytochemistry* **26**, 3297.
2. Jiménez, E. M., Portugal, M. E., Lira-Rocha, A., Soriano-Garcia, M. and Toscano, R. A. (1988) *J. Nat. Prod.* **51**, 243.
3. Misra, R., Pandey, R. C. and Dev, S. (1979) *Tetrahedron* **35**, 243.
4. Sharma, S. C., Tandon, J. S., Porter, B., Raju, M. S. and Wenkert, E. (1984) *Phytochemistry* **23**, 1194.
5. Achari, B., Chauduri, C., Saha, C. R., Dutta, P. K. and Pakrashi, S. C. (1990) *Phytochemistry* **29**, 3671.
6. Tori, K., Seo, S. and Tomita, Y. (1975) *Chem. Ind.* 434.
7. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.
8. Tori, K., Seo, S., Shimaoka, A. and Tomita,Y. (1975) *Tetrahedron Letters* 4227.
9. Cárdenas, Jorge (1992) *M. Sc. Thesis,* Fac. de Ciencias Químicas e Industriales, Universidad Autónoma del Estado de Morelos, México.
10. Maldonado, E., Flores, M. A., Salazar, B. and Ortega, A. (1994) *Phytochemistry* **37**, 1480.