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DITERPENES OF *SALVIA PRIONITIS*

LONG-ZE LIN*, GÁBOR BLASKÓ† and GEOFFREY A. CORDELL‡

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A.

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Key Word Index—*Salvia prionitis*; Labiatae; abietane type diterpenoids; salvinolone; salvinolactone; 4-hydroxy-sapriparaquinone; saprorthoquinone.

Abstract—From the roots of *Salvia prionitis* Hance two new diterpenoids, salvinolone and salvinolactone, and two 4,5-seco-5,10-*friedo*-abietane diterpenoids, 4-hydroxy sapriparquinone and saprorthoquinone, have been isolated and their structures elucidated on the basis of their ^{1}H and ^{13}C NMR spectra.

INTRODUCTION

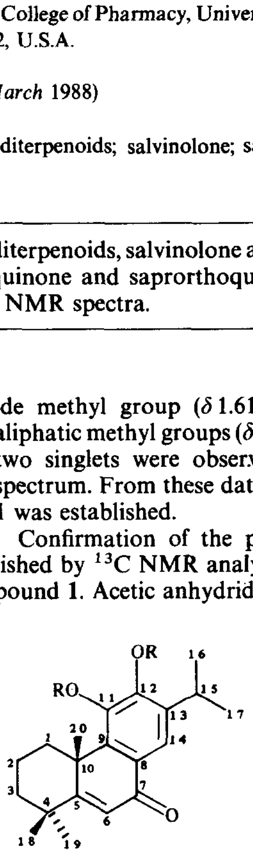
Salvia prionitis Hance is a rich source of diterpenoids. Previous phytochemical studies [1–4] have reported the isolation of four abietane type diterpenes, three 4,5-seco-5,10-*friedo*-abietane diterpenoids, and two tetracyclic diterpene derivatives. In this communication we report the isolation and structure elucidation of a new abietane diterpenoid, salvinolone (**1**), a new tetracyclic diterpene derivative, salvinolactone (**2**), and a new 4,5-seco-5,10-*friedo*-abietane diterpenoid, 4-hydroxy-sapriparaquinone (**3**), from the roots of *Salvia prionitis*, together with the unambiguous ^{1}H and ^{13}C NMR assignments of saprorthoquinone (**4**) [4] reisolated from this source, and the revision of the ^{13}C NMR assignments of aethiopinone (**5**) obtained previously from *Salvia aethiopis* [5].

RESULTS AND DISCUSSION

Dried and ground roots of *Salvia prionitis* were extracted with ethanol and after solvent evaporation the residue was partitioned between chloroform and water. The concentrated organic phase was repeatedly chromatographed on silica gel to afford three new diterpenes.

Salvinolone (**1**), mp 253–254°, $[\alpha]_D$ +35.8° (MeOH), displayed a molecular formula $C_{20}H_{26}O_3$ (M^+, *m/z* found 314.1871; calcd 314.1901), and its IR spectrum showed the presence of phenolic hydroxyl (ν_{max} 3420 cm^{-1}) and quinone methide moieties (ν_{max} 1640, 1560 cm^{-1}). The UV spectrum in MeOH [λ_{max} (log ε) 252 (4.04), 316 (3.62), 375 (3.40)] and in MeOH + NaOH [λ_{max} (log ε) 254 (4.02), 392 (3.76)] also supported the presence of these structural elements. The ^{1}H NMR spectrum (Table 1) indicated the presence of an isopropyl group (two methyl doublets at δ 1.18 and 1.20, $J = 6.8$ Hz, septet methine proton at δ 3.27, $J = 6.8$ Hz), one quinone methide methyl group (δ 1.61, *s*), and two non-equivalent aliphatic methyl groups (δ 1.71, *s*, and 1.36, *s*). In addition, two singlets were observed at δ 6.24 and 7.46 in the spectrum. From these data an abietane structure such as **1** was established.

Confirmation of the proposed structure was established by ^{13}C NMR analysis and derivatization of compound **1**. Acetic anhydride–pyridine treatment yielded a



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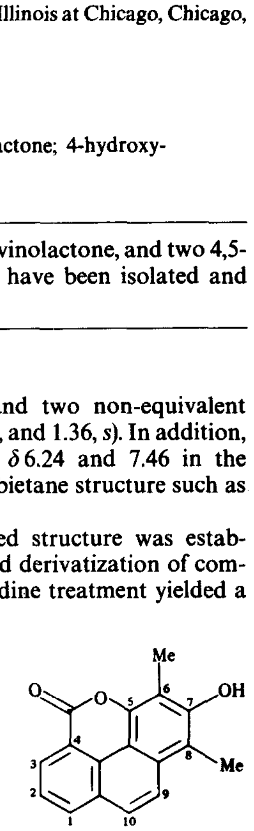
6 R = Ac



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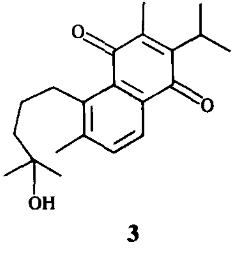

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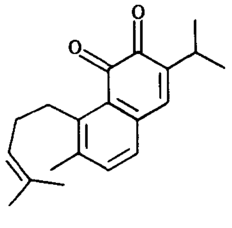

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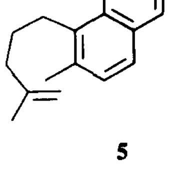

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*On leave from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.

†On leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.

‡Author to whom correspondence should be addressed.

diacetate **6**, confirming the presence of two phenolic hydroxy groups. The ^{1}H NMR spectrum (Table 1) of **6** showed significant shifts (to δ 6.52 and 8.15) for the aromatic/vinylic singlets. The remaining signals were essentially unaffected, with the exception of the isopropyl methine proton which was shifted to δ 2.96 indicating the close proximity of an acetyl group. Unambiguous assignment of the ^{13}C NMR spectra of **1** and **6**, was performed using APT, CSCM 1D [6] and selective INEPT [7] pulse programming sequences. In the CSCM 1D experiment an upfield or downfield carbon satellite of a proton signal is irradiated and magnetization is transferred to the attached carbon. In the *J*-modulated selective INEPT experiment, a particular proton is irradiated with a soft pulse resulting in magnetization transfer and a selective enhancement of carbon atoms three bonds away from the irradiated proton. CSCM 1D irradiation of the ^{13}C satellites of H-6, H-14 and H-15 in salvinolone (**1**) resulted in magnetization transfer to their corresponding carbon atoms appearing at δ 122.61, 114.13 and 24.58, respectively. Selective INEPT irradiation of H-14 enhanced the signals at δ 137.62, 147.49 and 183.84, of which the latter could be assigned to C-7. The two other quaternary carbon enhancements (C-9 and C-12) were distinguished when H-15 was irradiated resulting in enhancements at δ 114.13 (C-14) and δ 147.49 (C-12), thereby permitting the assignment of δ 137.62 as C-9. Polarization transfer from H-20 enhanced C-9 (δ 137.62), C-5 (δ 173.90) and C-1 (δ 33.86). Irradiation of H-6 resulted in enhancement of C-8 (δ 122.60), C-10 (δ 41.59) and C-4 (δ 37.58), and C-13 (δ 134.20) was assigned through irradiation of H-16 (and H-17. Irradiation of either H-18 or H-19 enhanced C-3 (δ 39.00) and C-5 (δ 173.90). The remaining unassigned quaternary carbon signal at δ 142.53 and the unassigned methylene carbon signal at δ 18.24 were established as C-11 and C-2, respectively. Details of the selective INEPT experiments are shown in Table 2, the complete assignments of the ^{13}C NMR spectra of salvinolone (**1**) and diacetyl salvinolone (**4**) are shown in Table 1.

Salvinolactone (**2**), mp 208–209°, displayed a $[M]^+$ at *m/z* 264.0770 for a molecular formula of $C_{17}H_{12}O_3$ (calcd 264.0790). Its IR and UV spectra showed the presence of phenanthridine and aromatic lactone moieties (see Experimental), and structure **2** was established by ^{1}H NMR and homonuclear COSY measurements. In the ^{1}H NMR spectrum, an ABX system (δ 8.49 *d*, $J = 8.3$ Hz,; 7.54 *dd*, $J_1 = 8.3$ Hz, $J_2 = 8.8$ Hz; and 7.45 *d*, $J = 8.8$ Hz) together with a well-separated AB system (δ 7.91 *d*, $J = 9.4$ Hz, and 7.86 *d*, $J = 9.4$ Hz) was observed due to the protons on rings A and B, respectively. Two methyl groups appeared at δ 2.41 and 2.74 and their locations were confirmed by a series of NOE experiments. Most important were the enhancement of the doublet at δ 7.86 on irradiation of the methyl signal at δ 2.47, and the lack of an NOE between

Table 1. ^{1}H and ^{13}C NMR spectral assignments of salvinolone (**1**) and *O,O*-diacetylsalvinolone (**6**).

	Salvinolone (**1**)*		*O,O*-Diacetyl-salvinolone (**6**)†	
Position	^{1}H	^{13}C	^{1}H	^{13}C
1	—	33.86	—	34.99
2	—	18.24	—	18.88
3	—	39.00	—	38.14
4	—	37.58	—	38.90
5	—	173.90	—	174.72
6	6.24 (*s*)	122.61	6.52 (*s*)	124.23
7	—	183.84	—	183.85
8	—	122.60	—	129.97
9	—	137.62	—	140.83
10	—	41.59	—	41.88
11	—	142.53	—	143.27
12	—	147.49	—	145.22
13	—	134.20	—	140.11
14	7.46 (*s*)	114.13	8.14 (*s*)	122.59
15	3.27 (*sep*., 6.8)	24.58	2.96 (*sep*., 6.8)	27.70
16	1.18[a] (*d*, 6.8)	22.76[a]	1.23[b] (*d*, 6.8)	22.88[b]
17	1.20[a] (*d*, 6.8)	22.58[a]	1.27[b] (*d*, 6.8)	22.73[b]
18	1.36 (*s*)	26.10	1.36 (*s*)	27.71
19	1.17 (*s*)	29.10	1.22 (*s*)	30.00
20	1.61 (*s*)	32.87	1.58 (*s*)	32.78
Ac	—	—	2.36 (s)	21.10
Ac	—	—	2.33 (s)	20.44
CO	—	—	—	167.62
CO	—	—	—	167.74

*Recorded in DMSO-d_6.

†Recorded in $CDCl_3$. Proton chemical shifts are reported as δ values (ppm) from internal TMS at 300 MHz. Signal multiplicity and coupling constants (Hz) are shown in parentheses. Carbon chemical shifts are reported as δ values (ppm) at 90.8 MHz.

[a, b]Interchangeable.

Table 2. Selective INEPT experiments on salvinolone (**1**) and diacetyl-salvinolone (**6**)

Salvinolone (**1**)*					Diacetyl-salvinolone (**6**)†				
Proton irradiated	δ	Carbon observed			Proton irradiated	δ	Carbon observed		
14-H	(7.46)	137.62 (C-9),	147.49 (C-12),	183.84 (C-7)	14-H	(8.14)	140.83 (C-9),	145.22 (C-12),	183.85 (C-7)
15-H	(3.27)	114.13 (C-14),	147.49 (C-12),		15-H	(2.96)	122.59 (C-14),	145.22 (C-12),	
20-H	(1.61)	137.62 (C-9),	173.90 (C-5),	33.86 (C-1)	20-H	(1.58)	140.83 (C-9),	174.72 (C-5),	34.99 (C-1)
6-H	(6.24)	122.60 (C-8),	41.59 (C-10),	37.58 (C-4)	6-H	(6.52)	129.97 (C-8),	41.88 (C-10),	38.90 (C-4)
16-H	(1.18)	134.20 (C-13),			16-H	(1.23)	140.83 (C-13),		
18-H	(1.36)	173.90 (C-5),	39.00 (C-3),		18-H	(1.36)	174.72 (C-5),	38.14 (C-3)	

*Recorded in DMSO-d_6.

†Recorded in $CDCl_3$. Chemical shifts values are reported in δ values (ppm).

the two methyl groups. The amount of salvinolactone (**2**) available precluded ^{13}C NMR investigation.

Two highly coloured crystalline compounds, 4-hydroxysapriparaquinone (**3**) and saprortoquinone (**4**) were also isolated from the dried roots of *Salvia prionitis*. These 4,5-seco-5,10-*friedo*-abietane diterpenoids are derived from the corresponding quinone methide abietane diterpenoids through acid-catalysed migration of the 10-methyl group to C-5 accompanied by fission of ring A [8].

4-Hydroxy-sapriparaquinone (**3**), mp 61–62°, exhibited IR and UV spectral data (see Experimental) suggesting the presence in the molecule of hydroxyl and α,β-unsaturated carbonyl groups, as well as a *p*-quinone chromophoric system. Low resolution EIMS showed two intense fragmentations at m/z 244 (100%) and 312 (80%). However, CIMS (CH_4 gas) indicated that the latter peak is not the molecular ion, but is rather a $[M^+ - 18]$ fragment, permitting establishment of the molecular formula as $C_{20}H_{26}O_4$.

The ^1H NMR spectrum of this compound (Table 3) displayed a doublet at δ 1.29 ($J = 6.7$ Hz) for two methyl groups and a septet at δ 3.37, indicating the presence of an isopropyl group. Two equivalent aliphatic methyl resonances were also observed at δ 1.25 as a six-proton singlet and an aromatic methyl signal appeared at δ 2.44. Three well-separated methylene proton absorptions were noted at δ 1.57, 1.72 and 3.14 whose coupling pattern was established from the homonuclear COSY spectrum. In the low field region of the ^1H NMR spectrum two *ortho* coupled aromatic protons were observed at δ 7.50 ($J = 7.8$ Hz) and δ 7.96 ($J = 7.8$ Hz), together with a singlet at δ 7.85 exchangeable with D_2O. The spectral data were consistent with the substitution pattern indicated in formula **3** of a *para*-quinone type of 4,5-seco-5,10-*friedo*-abietane diterpenoid, and the structure, 4-hydroxy-sapriparaquinone (**3**), was established by unambiguous ^{13}C NMR measurements using CSCM 1D [6] and selective INEPT [7] spectroscopic techniques. Details of selective INEPT experiments on compound **3** are shown in Table 4, and the complete assignment of the ^{13}C NMR spectrum of **3** is reported in Table 3.

Table 3. ^1H and ^{13}C NMR spectral assignments of 4-hydroxysapriparaquinone (**3**)*

C	^1H	^{13}C
1	3.13 (*dd*, 10.6, 8.6)	30.59
2	1.57 (*m*)	24.07
3	1.72 (*dd*, 16.1, 8.8)	44.15
4	—	71.01
5	—	142.92
6	7.50 (*d*, 7.8)	136.26
7	7.96 (*d*, 7.8)	125.40
8	—	133.24
9	—	126.23
10	—	144.62
11	—	183.24
12	—	153.16
13	—	126.42
14	—	184.46
15	3.37 (*sep.*, 6.7)	24.42
16	1.29 (*d*, 6.7)	19.80
17	1.29 (*d*, 6.7)	29.30
18	1.25 (*s*)	29.30
19	1.25 (*s*)	29.30
20	2.44 (*ss*)	20.29

*Recorded in $CDCl_3$. Proton chemical shifts are reported as δ values (ppm) from internal TMS at 300 MHz. Signal multiplicity and coupling constants (Hz) are shown in parentheses. Carbon chemical shifts are reported as δ values (ppm) at 90.8 MHz.

The fourth compound was identified on the basis of UV, IR, ^1H NMR, MS (see Experimental) evidence as saprorthoquinone (**4**), obtained previously from this plant source [4] and prepared by semi-synthesis as a rearranged derivative of 15-deoxyfuerstione [9]. Saprorthoquinone (**4**) is isomeric with aethiopione (**5**), differing only in the position of the double bond in the side chain ($\Delta^{3,4}$ in **4** and $\Delta^{4,19}$ in **5**). The ^{13}C NMR spectral data of **4** (Table 5) were not previously reported. Unambiguous ^{13}C NMR assignments for this compound were achieved using the previously mentioned APT, CSCM 1D [6] and selective INEPT [7] techniques. Selective INEPT irradiation experiments on compound **4** are summarized in Table 4. The complete assignment of ^1H and ^{13}C NMR spectra of saprorthoquinone (**4**) are shown in Table 5. As noted previously, saprorthoquinone (**4**) is an isomer of aethiopinone (**5**), differing only in the position of the double bond in the C-10 side chain. It would therefore be expected that the chemical shifts of the *o*-naphthoquinone carbons for **4** and **5** would correspond. However,

Table 4. Selective INEPT experiments on 4-hydroxy-sapriparaquinone (3) and saprorthoquinone (4)

4-Hydroxy-sapriparaquinone (3)		Saprorthoquinone (4)	
Proton irradiated δ	Carbon observed	Proton irradiated δ	Carbon observed
6-H (7.50)	144.62 (C-10), 133.24 (C-8), 20.29 (C-20)	6-H (7.04)	140.13 (C-10), 128.35 (C-8), 19.90 (C-20)
20-H (2.44)	144.62 (C-10), 136.24 (C-6)	20-H (2.39)	140.13 (C-10), 140.22 (C-6)
12-OH (7.83)	183.24 (C-11), 126.42 (C-13)	—	—
7-H (7.96)	184.46 (C-14), 142.92 (C-5), 126.23 (C-9)	7-H (7.36)	128.05 (C-14), 148.04 (C-5), 134.78 (C-9)
15-H (3.37)	184.46 (C-14), 153.16 (C-12), 19.80 (C-16, C-17)	15-H (3.02)	181.49 (C-12), 128.05 (C-14)
3-H_2 (1.72)	30.59 (C-1), 29.30 (C-18, C-19)	—	—
2-H_2 (1.57)	144.62 (C-10), 71.01 (C-4)	2-H_2 (2.19)	140.13 (C-10), 132.27 (C-4)
		1-H (3.05)	148.04 (C-5), 123.73 (C-3)

Recorded in $CDCl_3$. Chemical shifts values are in δ values (ppm).

Table 5. ^{1}H and ^{13}C NMR spectral assignments of saprorthoquinone (4) and proposed reassignment of the ^{13}C NMR spectrum of aethiopinone (5)

	Saprorthoquinone (4)*		Aethiopinone (5)†
Position	^{1}H	^{13}C	^{13}C
1	3.05 (*m*)	30.22	30.0
2	2.19 (*dd*, 15.6, 7.8)	27.46	26.9
3	5.30 (*t*, 8.2)	123.73	38.3
4	—	132.37	145.3
5	—	148.04	148.4
6	7.04 (*d*, 8.0)	140.22	140.0
7	7.36 (*dd*, 8.0, 1.9)	136.57	136.4
8	—	128.35	127.9
9	—	134.78	134.7
10	—	140.13	139.8
11	—	182.37	182.1
12	—	181.49	181.2
13	—	144.52	144.5
14	7.08 (*d*, 1.9)	128.05	127.9
15	3.02 (*sep*., 6.7)	26.84	26.8
16	1.17 (*d*, 6.7)	21.73	21.4
17	1.17 (*d*, 6.7)	21.73	21.4
18	1.78 (*s*)	25.74	22.3
19	1.63 (*s*)	17.59	110.0
20	2.39 (*s*)	19.90	19.7

*Spectra were recorded in $CDCl_3$. Proton chemical shift values are reported as δ values (ppm) from internal TMS at 300 MHz. Signal multiplicity and coupling constants (Hz) are shown in parentheses. Carbon chemical shifts are reported as δ values (ppm) at 90.8 MHz.

†Proposed reassigned carbon resonances based on data obtained from ref. [5].

in the published assignments for aethiopinone (**5**) [5] all but one of *o*-naphthoquinone skeleton carbon atoms are assigned differently from our values. Due to the accuracy of the methods applied for the unambiguous ^{13}C NMR assignment of saprorthoquinone (**4**), we suggest that the ^{13}C NMR data of aethiopinone (**5**) be revised as shown in Table 5.

All of the isolated compounds were evaluated in the P-388 and KB lymphocytic leukemia test systems *in vitro* according to established protocols [10, 11]. Salvinolone (**1**) exerted cytotoxic activity, ED_{50} 0.63 μg/ml, against the P-388 assay, however, in the KB assay it showed ED_{50} 7.73 μg/ml activity. Salvinolactone (**2**) and 4-hydroxy-sapriparaquinone (**3**) were found to be inactive in both the P-388 and KB test systems. Saprorthoquinone (**4**), however, displayed an ED_{50} = 4.80 μg/ml against the KB leukemia test system *in vitro*.

EXPERIMENTAL

Mps: uncorr. Mass spectra were determined at 70 eV. The ^{1}H NMR spectra were determined using TMS as internal standard. Homonuclear COSY spectra were obtained on a Varian XL-300 spectrometer with standard Varian pulse programs. All ^{13}C NMR spectra were measured at 90.8 MHz.

Plant material. The plant material of *Salvia prionitis* was collected in the Jiang-Xi Province, China in June, 1986 and identified by Dr Xiu-Lan Huang. A voucher sample is deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Isolation of diterpenoids. Dried and powdered roots of *Salvia prionitis* (11 kg) were extracted with EtOH (140 l), and the combined extracts evapd *in vacuo*. The residue was distributed between $CHCl_3$ (10 l) and H_2O (10 l), and the organic layer was washed with H_2O (2 × 2 l), dried and evapd to a residue (520 g) which was subjected to CC on silica gel (3 kg) eluting with $CHCl_3$. The fractions were evapd, examined by TLC and purified further through prep. TLC to yield salvinolone (**1**) (15 mg, 0.003%) and salvinolactone (**2**) (5 mg, 0.001%) 4-hydroxy-sapriparaquinone (**3**) (60 mg, 0.011%) and saprorthoquinone (**4**) (2.0 g, 0.38%) having the following physical and spectroscopic properties.

Salvinolone (**1**): mp 253–254°; $[\alpha]_D$ +35.8° (MeOH; *c* 0.12) IR ν_{max}^{KBr} cm^{-1}: 3420, 2970, 2965, 1640, 1560, 1475, 1370 UV λ_{max}^{MeOH} nm (log ε): 252 (4.04), 316 (3.63), 375 (3.40); $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 254 (4.02), 3.92 (3.76); ^{1}H and ^{13}C NMR: Table 1; MS, *m/z* (rel. int.): 314 $[M]^+$ (100), 299 (54), 271 (8), 258 (5), 244 (78), 232 (12), 229 (14), 215 (8), 203 (4). Mass measurement found 314.1871, calcd for $C_{20}H_{26}O_3$, 314.1901.

Salvinolactone (**2**): mp 208–209°; IR ν_{max}^{KBr} cm^{-1} 3450, 1735, 1700, 1620, 1600, 1570, 1350; UV λ_{max}^{MeOH} nm (log ε): 244 (4.02), 274 (3.99), 285 (4.10), 312 (3.64), 325 (3.71), 343 (3.61), 354 (3.62); ^{1}H NMR ($CDCl_3$): δ2.41 (*s*, 3H, 6-Me), 2.73 (*s*, 3H, 8-Me), 7.45 (*d*, *J* = 8.3 Hz, 1H, 1-H), 7.54 (*dd*, *J* = 8.3 Hz, *J* = 8.8 Hz, 1H, 2-H), 7.86 (*d*, *J* = 9.4 Hz, 1H, 9-H), 7.91 (*d*, *J* = 9.4 Hz, 1H, 10-H), 8.49 (*d*, *J* = 8.3 Hz, 1H, 3-H); MS, *m/z* (rel. int.): 264 $[M]^+$ (100), 235 (2), 208 (4), 184 (4), 178 (5), 152 (3), 132 (7); Mass measurement, found 264.0770, calcd. for $C_{17}H_{12}O_3$, 264.0790.

4-Hydroxy-sapriparaquinone (**3**): mp 61–62°; IR λ_{max}^{KBr} cm^{-1}: 3375, 3370, 2970, 1644, 1580, 1470, 1380, 1355, 1330, 1290 and 1255; UV λ_{max}^{MeOH} nm (log ε) 257 (4.23), 281 (4.13), 290 sh (4.08), 355 (3.53), 407 (2.78); $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 277 (4.31), 292 sh (4.13), 345 (3.38), 476 (3.34); ^{1}H and ^{13}C NMR; see Table 3; MS, *m/z* (rel. int.); 312 $[M-18]^+$ (80), 295 (11), 279 (5), 269 (12), 256 (36), 244 (100), 243 (60), 239 (9), 227 (9), 213 (10), 173 (6), 141 (7), 115 (12), 69 (78), 59 (61), 43 (38).

Saprorthoquinone (**4**): mp 97–98°; IR, UV and MS identical to reported values [4]; ^{1}H and ^{13}C NMR: see Table 5.

Diacetyl-salvinolone (**6**). Salvinolone (**1**) (8 mg, 0.025 mmol) was treated with Ac_2O (0.5 ml) in the presence pyridine (3 drops) at room temp. overnight. Work-up, including prep. TLC, yielded diacetyl-salvinolone (**3**) (7 mg, 80%) mp; 142–143; IR ν_{max}^{KBr} cm^{-1}: 3050, 1760, 1750, 1630, 1560, 1370; UV ν_{max}^{MeOH} nm (log ε) 260 (4.12), 274 sh (4.04); ^{1}H and ^{13}C NMR; see Table 1; MS, *m/z* (rel. int.) 398 $[M]^+$ (60), 356 (20), 314 (100), 299 (33), 287 (51), 258 (4), 245 (85), 232 (6).

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