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Rearranged abietane-type diterpenes from *Salvia dichroantha*

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Abstract

Three new irregular abietane-type diterpenes were isolated from the root of *Salvia dichroantha*. It was revealed by spectroscopic methods and chemical reaction that these compounds have a unique structure which contains a rearranged abietane skeleton. They were named dichroanal A and B and dichroanone and shown to be *rel*-(4a*S*,9*R*,9a*S*)-8-formyl-1,2,3,4,4a,9a-hexahydro-5,6,9-trihydroxy-7-isopropyl-1,1,4a-trimethylfluorene; 4a*S**-8-formyl-2,3,4,4a-tetrahydro-5,6-dihydroxy-7-isopropyl-1,1,4a-trimethyl-1*H*-fluorene and 4a*S**-2,3,4,4a-tetrahydro-6-hydroxy-7-isopropyl-1,1,4a-trimethyl-5,8(1*H*)-fluorenedione, respectively. *Salvia tomentosa* was found to contain seven known abietane-type diterpenes.

Keywords: *Salvia dichroantha*; *S. tomentosa*; Labiatae; Dichroanal A; Dichroanal B; Dichroanone; Abietane

1. Introduction

In a previous report on *Salvia* species, we described three clerodane-type diterpenoids from *S. splendens* (Hu, Kawazoe, & Takaishi, 1997). *Salvia* species are widespread in the tropical and temperate zones. A number of them are used as medicinal and culinary herbs. We have now examined *S. dichroantha* STAPF. and *S. tomentosa* MILLER collected in Turkey and, in this paper, we report on the isolation and structure elucidation of three novel diterpenes designated dichroanal A (**1**) and B (**2**) and dichroanone (**3**) each with a rearranged abietane skeleton along with one known compound, salvinolone (**4**), from *S. dichroantha* and seven known diterpenes from *S. tomentosa*. The six–five–six-membered ring system is a unique skeleton and only a few compounds with this structure have been reported from *Taiwania cryptomerioides* HAYATA (Lin, Fang, & Cheng, 1995).

2. Results and discussion

2.1. Salvia dichroantha

A methanol extract (138 g) of the dried roots of *S. dichroantha* was partitioned between ethyl acetate and water and the ethyl acetate-soluble part (51 g) was separated and purified by column chromatography to obtain compounds **1**–**4**.

Dichroanal A (**1**) was assigned the molecular formula $C_{20}H_{28}O_4$ by means of HR mass spectrometry. Its IR spectrum showed the presence of hydroxy (3432 cm^{-1}) and carbonyl (1651 cm^{-1}) groups. The ^{13}C NMR spectrum contained 20 carbon peaks made up of five methyls, three methylenes, three methines, eight quaternary carbons and one aldehyde carbon (δc 193.7). In the ^1H NMR spectrum, three singlet methyls (δ 1.13, 1.15, 1.19), an isopropyl moiety (δ 1.45, 1.44, each 3H, d, $J = 7.1$ Hz and δ 3.80, 1H, *sept*, $J = 7.1$ Hz) and one methine proton in the low-field (δ 5.25, dd, $J = 2.0$, 8.8 Hz) were observed. From the ^1H–^1H COSY spectrum, two partial structures were revealed, I: $-CH_2-CH_2-CH_2-$ and II: $>CH-CH-OH$. In the

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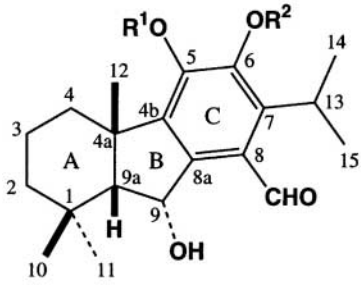

1 : $R^1 = R^2 = H$ **2** **3**

5 : $R^1 = CH_3$, $R^2 = H$

6 : $R^1 = CH_3$, $R^2 = Ac$

HMBC spectrum of compound **1**, the signal at δ1.82 (1H, d, $J = 8.8$ Hz, H-9a) for a proton in partial structure II had correlations with the carbon signals at δc 33.4 (C-1), 41.7 (C-2), 37.1 (C-4), 44.5 (C-4a), 136.2 (C-4b), 23.4 (C-12), 74.4 (C-9) and 143.4 (C-8a). Additionally, we could detect long range correlations of the proton signal at δ 1.13 (3H, s, H-12) with the carbon signals at δc 37.1 (C-4), 44.5 (C-4a), 136.2 (C-4b) and 64.3 (C-9a) and of the proton signal at δ 1.15 (3H, s, H-11) with the carbon signals at δc 33.4 (C-1), 41.7 (C-2), 64.3 (C-9a) and δc 22.3 (C-10). From these facts, partial structures I and II were combined across two quaternary carbons (δc 33.4 and 44.5) and the structure of the A ring in compound **1** was confirmed to be as shown in formule **1**. The presence of one benzene ring was deduced from the remaining carbon signals (δc 136.2, 144.8, 142.0, 138.2, 124.9, 143.4). Although carbon signals assignable to our aromatic ring were observed, no aromatic proton signals were found in ^{1}H NMR and thus the aromatic ring was hexa-substituted. Long range correlations were observed from the proton signals at δ 3.80 (1H, *sept*, $J = 7.1$ Hz, H-13) to the carbon signals at δc 142.0 (C-6), 138.2 (C-7) and 124.9 (C-8), and from the proton signal at δ 10.40 (1H, s, CHO) to the carbon signals at δc 143.4 (C-8a), 136.2 (C-4b) and 124.9 (C-8). Thus from the NMR data, the presence of a benzene ring bearing formyl, isopropyl and two hydroxy groups was confirmed. The remaining proton signal at δ 5.25 had a long-range correlation with the carbon signal at δc 136.2 (C-4b) in the HMBC spectrum. From these facts, we deduced that the structure of **1** had a six–five–six-membered ring. The positions of the substituents on the benzene ring were established by nOe experiments. The nOe's between the isopropyl moiety and the formyl group were detected in dichroanal A (**1**). Compound **1** was treated with trisilyldiazomethane to give the monomethyl ether **5**. An nOe between the methoxy group and C-12 of **5** indicated the presence of a methoxy group at C-5. Furthermore, when compound **5** was acetylated by acetic anhydride to give monoacetate **6**, an nOe was observed between the acetoxy group and both the methoxy and isopropyl group of **6**. On the basis of this finding the acetoxy and isopropyl groups were assigned to C-6 and C-7, respectively. Consequently, the formyl group had to be at C-8. The relative configuration (C-4a, 9a and 9) was clarified by nOe correlations (Fig. 1). The structure of dichroanal A was thus elucidated as *rel*-(4a*S*, 9*R*, 9a*S*)-8-formyl-1,2,3,4,4a,9a-hexahydro-5,6,9-trihydroxy-7-isopropyl-1,1,4a-trimethylfluorene.

Dichroanal B (**2**), $C_{20}H_{26}O_3$, showed a carbonyl band at 1646 cm^{-1} and a hydroxy band at 3402 cm^{-1} in the IR spectrum. Its ^{1}H NMR spectrum was very similar to that of compound **1** except for the presence of a double bond proton at δ 7.58 (1H, s) in compound **2** and a hydroxy methine proton at δ 5.25 in compound **1**. The chemical shifts in the ^{13}C NMR spectrum were also analogous to those of compound **1** except for the double bond carbons (δc 120.9 and 167.0) in compound **2** and methine carbons (δc 74.4

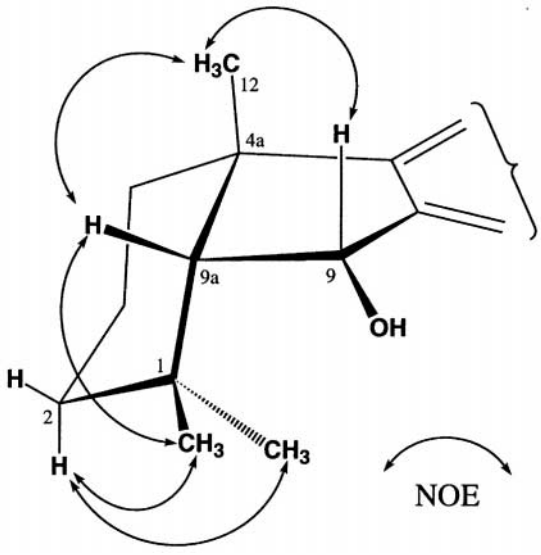

Fig. 1.

and 64.3) in compound **1**. Furthermore, an nOe was observed between the aldehyde proton and H-9 of the isopropyl methyl group. From these facts and a consideration of the molecular formula, dichroanal B was obviously a dehydrated form of **1**. In the HMBC spectrum, correlations from the proton signal at δ 7.58 (H-9) and the carbon signals at δc 51.1 (C-4a), 139.3 (C-4b), 120.6 (C-8) and 167.0 (C-9a) were observed. Thus dichroanal B is established as 4a*S**-8-formyl-2,3,4,4a-tetrahydro-5,6-dihydroxy-7-isopropyl-1,1,4a-trimethyl-1*H*-fluorene.

Dichroanone (**3**) was assigned the molecular formula $C_{19}H_{24}O_3$. The IR spectrum showed carbonyl and hydroxy bands at 1627 and 3324 cm^{-1} and the UV spectrum indicated the presence of a quinone moiety. Its ^{13}C NMR spectrum showed three olefins and two shielded carbonyl carbons. In the ^1H–^1H COSY spectra, an isopropyl moiety, three singlet methyls and $–CH_2–CH_2–CH_2–$ unit were confirmed, cf. dichroanal A (**1**). The ^1H NMR spectrum of **3** was analogous to that of **1** except for the signal at δ 6.45 and 7.31 in **3** and δ 10.40 in **1**. From these facts, it was considered that **3** had a similar structure to **1** but lacked an aromatic ring and formyl group. In the HSQC spectrum, one singlet proton at δ 7.31 was assigned to a hydroxy proton because it had no correlations with any carbon. The HMBC spectrum revealed that the A ring was as same as in **1**. The singlet proton signal at δ 6.45 showed correlations with the carbon signals at δc 37.1 (C-1), 55.5 (C-4a), 148.0 (C-4b), 185.8 (C-8) and 178.3 (C-9a) and the proton signal at δ 7.31 (OH) with δc 177.3 (C-5), 152.6 (C-6) and 123.0 (C-7). From these facts, the hydroxy group was placed at C-6. Consequently, dichroanone was also a rearranged abietane having a five-membered ring i.e. 4a*S**-2,3,4,4a-tetrahydro-6-hydroxy-7-isopropyl-1,1,4a-trimethyl-5,8(1*H*)-fluorenedione.

Compound **4** showed the characteristic NMR spectra of an abietane-type diterpene and was identified by spectral data comparison as salvinolone (Lin, Blaskó, & Cordell, 1989).

Dichroanal A and B, and dichroanone have a unique, six–five–six-membered ring system which prior to this study was found in only a few compounds reported from *Taiwania cryptomerioides* HAYATA (Lin et al., 1995).

2.2. Salvia tomentosa

The MeOH extract of the root of *S. tomentosa* was partitioned between ethyl acetate and water. The ethyl acetate-soluble part was purified by repeated chromatography and yielded seven compounds **7–13**. The spectroscopic data showed that they were all abietanes. The structure of each compound was established by comparison of its spectral data with those reported in the literature for horminone (**7**) (Jonathan, Che, Pezzuto, Fong, & Farnsworth, 1989), sugiol (**8**) (Chang et al., 1990), 7α-acetoxyroyleanone (**9**) (Matsumoto & Harada, 1979), rosmanol (**10**) (González, Andrés, Herrera, Luis, & Ravelo, 1989), 8,11,13-abietatriene-18-oic acid (**11**) (Kutney & Dimitriadis, 1982), salviol (**12**) (Hayashi, Handa, Ohashi, & Kakisawa, 1971) and 12-methoxycarnosic acid (**13**) (Al-Hazimi, Miana, & Deep, 1987).

3. Experimental

3.1. General

^1H and ^{13}C NMR: 400 and 100 MHz, respectively. Other spectroscopic procedures were the same as described in Hu et al. (1997).

3.2. Plant material

The root of *Salvia dichroantha* STAPF. and *Salvia tomentosa* MILLER ware collected in 1994, Turkey. Herbal specimens (94B129 and 94A058) are deposited in the herbarium of Kyoto University and Gazi University.

3.3. Extraction and fractionation

S. dichroantha: The MeOH extract from 1.33 kg of dried material (138 g) was partitioned between EtOAc and water and the EtOAc-sol. part (51 g) loaded onto a silica gel column and eluted with *n*-hexane–EtOAc to obtain two frs (1 and 2). Fr. 1 was fractionated by HPLC [Shodex GPC H-2001 ($CHCl_3$), YMC Pac Si60 (*n*-hexane–EtOAc)] to yield dichroanal A (**1**, 4.6 mg). Fr. 2 was purified in the same manner to give dichroanal B (**2**, 14 mg), dichroanone (**3**, 6.9 mg) and salvinolone (**4**, 20 mg). *S. tomentosa*: Dried root (3.9 kg) was cut and extracted with MeOH and the MeOH extract (286 g) partitioned with EtOAc and water. The EtOAc-sol. part (69 g) was fractionated by CC, HPLC and PLC repeatedly to obtain compounds **7** (29 mg), **8** (1.2 mg), **9** (5.9 mg), **10** (3.1 mg), **11** (33 mg), **12** (19 mg) and **13** (6.8 mg).

3.3.1. Dichroanal A (**1**)

Amorphous yellowish powder, $[\alpha]_D^{25}$ +4.3° (dioxane, *c* 0.23). HREI-MS: *m*/*z* 332.1971 $[M]^+$, calcd for $C_{20}H_{28}O_4$, 332.1988; IR ν_{max}^{KBr} cm^{-1}: 3432, 2928, 1651, 1562, 1455, 1279; UV λ_{max} nm (log ε): 240 (2.8), 281 (2.5), 320 (2.3); ^1H and ^{13}C NMR: Tables 1 and 2.

3.3.2. Dichroanal B (**2**)

Amorphous yellowish powder, $[\alpha]_D^{25}$ −8.2° (dioxane, *c* 0.86). HREI-MS: *m*/*z* 314.1854 $[M]^+$, calcd for

Table 1. ^{1}H NMR data for compounds **1**–**3**

H	**1**[a]	**2**[b]	**3**[c]
2	1.27, 1H, m	1.12, 1H, m	ca. 1.1, 1H, m
	1.54, 1H, ddd, J = 2.9,2.9,10.4 Hz	1.57, 1H, m	1.71, 1H, ddd, J = 2.5,2.5,7.5 Hz
3	ca. 1.8, 1H, m	1.94, 1H, m	1.93, 1H, m
	1.27, 1H, m	ca. 1.5, 1H, m	ca. 1.6, 1H, m
4	ca. 1.8, 1H, m	ca. 1.2, 1H, m	ca. 1.1, 1H, m
	2.21, 1H, m	2.91, 1H, br d, J = ca. 13 Hz	2.38, 1H, br dd, J = ca.2, 13 Hz
9	5.25, 1H, dd, J = 8.8, 2.0 Hz	7.58, 1H, s	6.45, 1H, s
9a	1.82, 1H, d, J = 8.8 Hz	–	–
10	1.19, 3H, s	1.24, 3H, s	1.24, 3H, s
11	1.15, 3H, s	1.30, 3H, s	1.29, 3H, s
12	1.13, 3H, s	1.72, 3H, s	1.46, 3H, s
13	3.80, 1H, *sept*, J = 7.1 Hz	4.46, 1H, *sept*, J = 7.1 Hz	3.22, 1H, *sept*, J = 7.0 Hz
14	1.44, 3H, d, J = 7.1 Hz	1.65, 3H, d, J = 7.1 Hz	1.24, 3H, d, J = 7.0 Hz
15	1.45, 3H, d, J = 7.1 Hz	1.65, 3H, d, J = 7.1 Hz	1.25, 3H, d, J = 7.0 Hz
8-CHO	10.40, 1H, s	10.94, 1H, s	–
9-OH	5.24, 1H, d, J = 2.0 Hz	–	–
6-OH	[c]	[c]	7.31, 1H, s

[a]In $CDCl_3$.
[b]In pyridine-d_5.
[c]Overlapped.

$C_{20}H_{26}O_3$, 314.1882; IR ν_{max}^{KBr} cm^{-1}: 3402, 2931, 1646, 1555, 1433, 1269; UV λ_{max} nm (log ε): 259 (4.1), 265 (4.1), 295 (3.9), 343 (3.7); ^{1}H and ^{13}C NMR: Tables 1 and 2.

3.3.3. Dichroanone (**3**)

Amorphous red powder. $[\alpha]_D^{25}$ –99.3° (dioxane, c 0.67). HREI-MS: m/z 300.1731 $[M]^+$, calcd for $C_{19}H_{24}O_3$, 300.1725; IR ν_{max}^{KBr} cm^{-1}: 3324, 2958, 1627, 1520, 1457, 1288; UV λ_{max} nm (log ε): 253 (4.0), 332 (4.0); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Table 2. ^{13}C NMR data for compounds **1**–**3**

C	**1**[a]	**2**[b]	**3**[a]
1	33.4	35.8	37.1
2	41.7	43.0	43.6
3	20.1	19.7	19.2
4	37.1	36.3	37.5
4a	44.5	51.1	55.5
4b	136.2	139.3	148.0
5	144.8	142.6	177.3
6	142.0	142.6	152.6
7	138.2	138.9	123.0
8	124.9	120.6	185.8
8a	143.4	140.8	149.0
9	74.4	120.9	118.1
9a	64.3	167.0	178.3
10	22.3	25.6	24.9
11	34.2	31.5	31.0
12	23.4	20.3	20.3
13	27.1	27.0	24.1
14	22.3	22.7	20.2
15	22.0	22.6	20.2
CHO	193.7	191.7	–

[a]In $CDCl_3$.
[b]In pyridine-d_5.

3.4. Methylation of dichroanal A (**1**) followed by acetylation

Dichroanal A (**1**, trace amount) was dissolved in CH_2Cl_2 (1 ml) with a few drops of EtOH. A few drops of trimethylsilyldiazomethane were added at room temp. and allowed to stand for 30 min. The solvent was evaporated under reduced pressure to obtain monomethyl ether, **5**. Next, **5** was dissolved in pyridine and Ac_2O (each 1 ml). After 1 h, MeOH was added and the of solvent received in vacuo to yield acetate, **6**.

3.4.1. Compound **5**

Amorphous red powder. HREI-MS: m/z 346.2144 $[M]^+$, calcd for $C_{21}H_{30}O_4$, 346.2144; ^{1}H NMR ($CDCl_3$): δ 10.51 (1H, s, 8-CHO), 5.26 (1H, dd, J = 2.5, 10.0 Hz, H-9), 4.79 (1H, d, J = 2.5 Hz, 9-OH), 3.85 (3H, s, 5-OCH_3), 3.82 (1H, *sept*, J = 7.0 Hz, H-13), ca. 2.3 (1H, br d, J = ca. 11 Hz, H-4), 1.46, 1.45 (each 3H, d, J = 7.0 Hz, H-14, 15), 1.18 (3H, s, H-10), 1.14 (3H, s, H-11), 1.11 (3H, s, H-12).

3.4.2. Compound **6**

Amorphous red powder. HREI-MS: m/z 388.2230 $[M]^+$, calcd for $C_{23}H_{32}O_5$, 388.2250; ^{1}H NMR ($CDCl_3$): δ 10.55 (1H, s, 8-CHO), 5.31 (1H, dd, J = 2.6, 10.2 Hz, H-9), 4.72 (1H, br s, 9-OH), 3.82 (3H, s, 5-OCH_3), 3.62 (1H, *sept*, J = 7.1 Hz, H-13), 2.38 (3H, s, 6-CH_3CO), ca. 2.3 (1H, m, H-4), 1.81 (1H, d, J = 10.2, H-9a), 1.40, 1.39 (each 3H, d, J = 7.1 Hz,

H-14, 15), 1.17 (3H, s, H-10), 1.14 (3H, s, H-11), 1.12 (3H, s, H-12).

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