seco-Hinokiol, a New Abietane Diterpenoid from *Rosmarinus officinalis*

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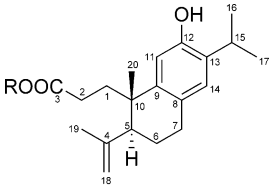
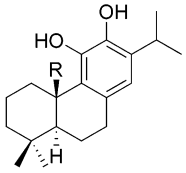
seco-Hinokiol (**1**), a new abietane diterpenoid, has been isolated from the leaves of *Rosmarinus officinalis*. Its structure was elucidated on the basis of extensive spectroscopic analysis. To our knowledge, this is the fourth report of a *seco*-abietane having been isolated. In addition, methyl carnosate (**4**) was synthesized from carnosic acid (**3**), and detailed NMR spectroscopic data are provided to clarify previous literature reports.

Rosemary, *Rosmarinus officinalis* L. (Lamiaceae), is a well-known herb used for centuries as a spice and in folk medicine around the world.[1,2] The antioxidant properties of rosemary have been well documented, and extracts of its leaves are used to prevent the oxidation of fats and the resulting formation of objectionable compounds with unpleasant flavors.[3,4] Numerous phenolic abietane diterpenes have been isolated from rosemary including the potent antioxidant carnosic acid.[5−7] There are several reports that have established carnosic acid as the major phenolic diterpenoid present in fresh rosemary leaves.[8−13] In our continued search for novel antioxidants from the Lamiaceae, we have re-examined the leaves of rosemary.

The concentrated acetone extract of the rosemary needles was treated with a 5% $NaHCO_3$ solution followed by vacuum filtration. The resulting filtrate was treated with H_3PO_4 and the precipitate collected on a Buchner funnel. This process was repeated, followed by vacuum-liquid chromatography (VLC) and reversed-phase HPLC purification, to yield carnosic acid (**3**) and the new abietane diterpenoid *seco*-hinokiol (**1**).



1 R = H
2 R = CH_3



3 R = COOH
4 R = COOMe

Compound **1**, obtained as a colorless powder, was analyzed using both negative-ion high-resolution ESIMS and positive-ion ESIMS. Negative-ion HRESIMS analysis resulted in a molecular ion peak at *m/z* 315.1946 $[M - H]^-$ (calculated for $C_{20}H_{27}O_3$, 315.1966), while positive-ion ESIMS analysis led to both $[M + Na]^+$ (*m/z* 339.2) and $[M + K]^+$ (*m/z* 355.3) ions. The combined negative- and positive-ion ESIMS data suggested a molecular formula of $C_{20}H_{28}O_3$ and seven sites of unsaturation. Its IR spectrum showed a strong absorption band at 1707 cm^{-1}, indicating a carbonyl functionality.

The ^1H NMR spectrum of compound **1** indicated the presence of two olefinic protons [δ 4.74 (s, 1H) and 4.94 (s, 1H)]; two aromatic protons [δ 6.73 (s, 1H) and 6.81 (s, 1H)]; an isopropyl group [δ 1.18 (d, 3H), 1.19 (d, 3H), and 3.22 (sept., 1H, $J = 7.0$ Hz)]; an olefinic methyl [δ 1.80 (s, 3H)]; and one additional methyl singlet [δ 1.16 (3H)]. The ^{13}C and DEPT NMR spectra indicated the presence of 20 carbons: four methyls, five methylenes, four methines, and seven quaternary. The ^{13}C NMR spectrum indicated the presence of two olefinic carbons at δ 147.2 (C-4) and 113.7 (C-18), one carboxylic carbonyl at δ 174.3 (C-3), and six aromatic carbons between δ 112.4 and 152.8, and the ^1H and ^{13}C NMR data were typical of an abietane diterpenoid and quite similar to those of formosanic acid.[5,14]

Careful analysis of the $^1H-^1H$ COSY spectroscopic data confirmed the presence of an isopropyl group with couplings between H-15 and the methyl signals at δ 1.18 (H-17) and 1.19 (H-16), and two additional coupling systems were observed: one between the methylene protons H-1 and H-2 and the other between the methine proton H-5 and the methylenes H-6 and H-7. The combined $^1H-^{13}C$ HSQC and $^1H-^{13}C$ HMBC spectral data helped to establish the structure of compound **1**. Correlations between H-5 and C-4, C-6, C-10, C-18, C-19, and C-20 were instrumental in establishing the proposed structure. The correlations between H-5 and C-4, C-18, and C-19 firmly established the positioning of the vinylic methyl olefin group. In addition, correlations were observed between H-20 and C-1 as well as between H-2 and C-1 and C-3, helping to establish the location of the carboxylic acid as well as its relationship to the B ring. Orienting the aromatic hydroxyl and isopropyl groups onto ring C was accomplished primarily by HMBC correlations from H-11, H-15, H-16, and H-17 to C-13. In addition, correlations were observed between H-14 and C-12, between H-11 and C-8, and between H-7 and C-8 and C-9. Furthermore, confirmation of the presence of a carboxylic functional group was made by methylating **1** with CH_2N_2 to produce the methyl ester **2**. ^1H NMR analysis of compound **2** indicated the presence of a methyl singlet at δ 3.55 (3H) corresponding to OMe. In addition, negative-ion ESIMS analysis of **2** gave a molecular weight of 329.0, corresponding to $[M - H]^-$.

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data and HMBC Correlations for Compound **1** (acetone-d_6) and ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data for Compound **4** ($CDCl_3$)

	1			**4**	
position	δ_C[a]	δ_H, mult (J in Hz)	HMBC	δ_H, mult (J in Hz)	δ_C[a]
1	34.8 t	1.97[b], 2.01[b]	2, 20	3.30 m	35.0 t
2	28.6 t	1.82[b], 2.24 ddd (4.7, 12.2, 15.4)			20.4 t
3	174.3 s		2		42.1 t
4	147.2 s		5, 19		34.4 s
5	47.0 d	2.46 dd (2.6, 11.6)	7, 18, 19		54.0 d
6	25.1 t	1.88[b], 1.89[b]	5, 7		19.3 t
7	29.0 t	2.66[b], 2.71[b]		2.78 m, 2.81 m	31.7 t
8	127.7 s		7, 11		128.5 s
9	141.1 s		7, 20		122.1 s
10	40.4 s		5, 20		49.1 s
11	112.4 d	6.73 s			142.1 s
12	152.8 s		14		141.7 s
13	133.4 s		11, 15, 16, 17		133.4 s
14	126.5 d	6.81 s	15	6.54 s	119.2 d
15	26.7 d	3.22 sept. (7.0)	16, 17	3.19 sept. (7.0)	27.2 d
16	22.0 q	1.19 d (7.0)	15, 17	1.21 d (7.0)	22.6 q
17	22.2 q	1.18 d (7.0)	15, 16	1.18 d (7.0)	22.2 q
18	113.7 t	4.74 br s, 4.94 br s	5, 19	0.99 s	32.7 q
19	22.4 q	1.80 s	5, 18	0.80 s	21.8 q
20	27.6 q	1.16 s	5		178.9 s
OMe				3.66 s	52.2 q

[a] Multiplicity deduced from the DEPT spectrum. [b] Signal overlap prevented coupling constant determination.

Final structural confirmation was accomplished by comparing chemical shift data reported in the literature for formosanic acid with those of compound **1**.[14] Formosanic acid is identical to **1** except for the arrangement of the aromatic hydroxyl and isopropyl functional groups. All nonaromatic ^{13}C NMR signals were identical to those of formosanic acid. The aromatic ^{13}C NMR signals were similar to those of hinokiol and ferruginol, suggesting an identical aromatic ring structure.[15−18] Therefore, compound **1** was assigned as 12-hydroxy-3,4-secoabieta-4(18),8,11,-13-tetraen-3-oic acid and given the trivial name *seco*-hinokiol, based on the assumption that it could have been formed from hinokiol following both oxidation and elimination resulting in cleavage of the A ring. Last, the absolute configuration was confirmed to be that shown by comparing optical rotation data of **1** with those reported in the literature for both (+)-hinokiol and (+)-hinokione, for which complete stereoseletive and enantioselective synthesis has been performed.[19]

A significant amount of literature information is available regarding the isolation and identification of methyl carnosate (**4**).[20−23] However, some of the ^{1}H and ^{13}C NMR data reported appear to be incorrect. For example, the purification of **4** from *Salvia officinalis* L. (Lamiaceae) has been reported[21] along with its ^{1}H NMR data. The authors identified **4** based solely on the presence of an additional methyl at δ 1.10 and assigned this signal to the OMe of the methyl ester. However, the methyl signal of a methyl ester would be expected to appear around δ 3.6. A second report on the isolation of **4** from a *Salvia* species incorrectly reported the ^{13}C NMR data for this compound, and it is believed the authors may have actually isolated 12-methoxycarnosic acid.[20] In fact, a latter publication by the same authors reported the isolation of 12-methoxycarnosic acid from the same species with identical NMR data.[22] In addition, no reports exist on the methylation of carnosic acid (**3**) directly to methyl carnosate (**4**), which would confirm its structure unequivocally; spectroscopic data for carnosic acid (**3**) have been very well established.[5] We have converted carnosic acid (**3**) into its corresponding methyl ester (**4**) using CH_2N_2, and the unambiguous ^{1}H and ^{13}C NMR data of **4** are presented in Table 1.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Autopol III automatic polarimeter (Rudolph Research, Flanders, NJ). UV and IR spectra were recorded on a Shimadzu 2401-PC and a Perkin-Elmer 1600 FT-IR instrument, respectively. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL Eclipse 400-MHz NMR spectrometer (Peabody, MA), equipped with a Silicon Graphics Indigo workstation and DELTA NMR software, using either $CDCl_3$ or acetone-d_6 as the solvent. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in Hz. Low-resolution ESIMS were obtained on a Fisons VG Platform quadrupole mass spectrometer, and high-resolution ESIMS were obtained on an Agilent LC/MSD TOF. All ESIMS and HRESIMS analyses were performed on samples prepared at 0.1 mg/mL in MeOH.

Plant Material. Needles of *Rosmarinus officinalis* (rosemary) were purchased from a Moroccan supplier in March 2001 and constitute Hauser lot number A102910 and voucher number 0103200913.

Analytical HPLC Analysis. The HPLC system consisted of a model L-7100 pump, a model L-7250 autosampler equipped with a 100 μL loop, and a model L-7450A diode array detector (Hitachi Instruments, Inc., Fremont, CA). The column was a 4.6 mm × 15 cm Zorbax SB-C_{18}, 5 μm (Mac-Mod Analytical, Chadds Ford, PA). The analysis was run at 1.5 mL/min with a 65:35 mixture of acetonitrile and water containing 0.1% phosphoric acid with a monitoring wavelength of 230 nm. Spectral data were obtained over the range of 215−500 nm.

Semipreparative HPLC. A Waters Delta Prep 4000 system was used equipped with a Waters 2487 detector and 5 mL injection loop. The column was a 2.5 × 10 cm NovaPak C_{18} cartridge installed in a RCM 25 × 20 cm radial compression holder (all Waters Chromatography, Milford, MA). The system was operated at a flow rate of 30 mL/min and utilized a gradient starting at 40/60 acetonitrile/0.1% TFA and going to 45/55 after 16 min.

Extraction and Isolation. Dried rosemary needles (100 g) were extracted with 500 mL of acetone at 40 °C for 1 h.

The extract was filtered from the needles, the acetone was evaporated to 50 mL volume, and 150 mL of 5% $NaHCO_3$ was added. Insoluble material was removed by vacuum filtration, and the basic filtrate was treated with H_3PO_4 to achieve pH 2.2. The crude mixture of carnosic acid (**3**: 42%) and **1** (15%) was collected on a Buchner funnel. After drying, 2.5 g of this crude material was subjected to a second basic dissolution with 150 mL of 2% $NaHCO_3$, followed by filtration using a Celite bed and acidification of the filtrate with HCl. The filter cake was not dried but dissolved in 30 mL of methanol, and 25 mL of water was added, forming an emulsion. This suspension was loaded on a VLC column, prepared using a 4.5 cm, 60 mL glass sintered filter, packed with 30 mL of Bakerbond 40 μm flash chromatography medium (Baker Chemicals, Phillipsburg, PA). After loading, the column was eluted with 50 mL of 50% (v/v) methanol and 100 mL each of 55, 60, 65, and 70% methanol. The unknown **1** was found in the 70% methanol fraction, and therefore a second 100 mL aliquot of 70% methanol was used and the two were combined. Evaporation of the combined 70% fractions gave off-white solids (300 mg) containing approximately 170 mg of **1** and approximately 130 mg of **3**. About 100 mg of these solids was dissolved in 5 mL of methanol, and four 1 mL injections were performed on the semipreparative HPLC using the conditions described above. Compound **1** eluted before **3** at about 8 min, and the combined fractions were evaporated to yield 46 mg of **1**.

***seco*-Hinokiol (1):** colorless powder; $[\alpha]^{25}_D$ +77.2° (*c* 0.86, MeOH); IR (film, KBr) ν_{max} 2882, 1707, 1509, 1417, 1194, 1013, 894 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz), see Table 1; HRESIMS negative-ion mode, *m/z* 315.1946 $[M - H]^-$ (calcd for $C_{20}H_{27}O_3$, 315.1966), ESIMS positive-ion mode, *m/z* 339.2 $[M + Na]^+$, 355.3 $[M + K]^+$.

Methylation of *seco*-Hinokiol (1). The experiment was performed using a Sigma-Aldrich diazomethane generation kit. The CH_2N_2 was prepared according to manufacturer's protocol, collected in ether, and stored until needed. An aliquot (1.8 mL) of the CH_2N_2-ether solution was pipetted into a 2 mg/mL methanol solution (2.7 mL) of compound **1**. The reaction mixture was placed in a fume hood overnight to allow the volatiles to evaporate, yielding 5.6 mg of the methylated derivative **2**: colorless powder; ^1H NMR (acetone-d_6, 400 MHz) δ 6.81 (1H, s, H-14), 6.73 (1H, s, H-11), 4.94 (1H, br s, H-18), 4.72 (1H, br s, H-18), 3.55 (3H, s, OMe), 3.21 (1H, sept., J = 7.0 Hz, H-15), 2.71 (1H, m, H-7), 2.67 (1H, m, H-7), 2.45 (1H, dd, J = 2.6, 11.6 Hz, H-5), 2.25 (1H, ddd, J = 4.7, 12.2, 15.4 Hz, H-2), 1.78 (3H, s, H-19), 1.19 (3H, d, J = 7.0 Hz, H-16), 1.18 (3H, d, J = 7.0 Hz, H-17), 1.16 (3H, s, H-20); ESIMS negative-ion mode, *m/z* 243.3, 314.4, 329.0 $[M - H]^-$; ESIMS positive-ion mode, *m/z* 352.9 $[M + Na]^+$.

Methylation of Carnosic Acid (3). Diazomethane generation was performed as described above. A 1.8 mL sample of this CH_2N_2-ether solution was added to a 4 mg/mL methanol solution (2 mL) of compound **3**. The reaction mixture was placed in a fume hood overnight to allow the volatiles to evaporate, yielding 8.3 mg of methyl carnosate (**4**): light yellow powder; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESIMS, *m/z* 345.1 $[M - H]^-$.

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