Quinone Derivatives by Chemical Transformations of 16-Hydroxycarnosol from *Salvia* Species

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The known diterpenes 12,16-epoxycarnosol (2), isotanshinone II (6), and (+)-neocryptotanshinone (8) were obtained by partial synthesis from 16-hydroxycarnosol (1), a C-16 hydroxylated abietatriene diterpene isolated in relative abundance from the aerial part of *Salvia mellifera* Greene. The physical and spectroscopic data of these semisynthetic diterpenes were identical to those given for the natural ones in the literature. These abietane diterpenes have very interesting biological activities and the semisynthetic approach described here represents an alternative to obtain them from other major diterpenes isolated from *Salvia* species. Additionally, seven new semi-synthetic diterpene analogues, 11,14-dioxo-12,16-epoxy-8,12-abietadien-20,7β-olide (3), 11,14-dioxo-12,16-epoxy-8,12,15(16)-abietatrien-20,7β-olide (4), 15,16-didehydro-12,16-epoxycarnosol (5), 1-o xoisotanshinone II (7), 16-hydroxycolumbardione (9), 12,16-diacetoxycolumbaridione (10), and 14-methoxy-12,16-epoxycarnosol (13), were obtained from 1. The structures of the new compounds were established based on their spectroscopic data.

Key words semisynthesis; *Salvia mellifera*; abietane diterpene; Lamiaceae; quinone derivative; 16-hydroxycarnosol

Results and Discussion

In the course of our work on the partial synthesis of diterpenes related to tanshinones, we thought that 16-hydroxycarnosol (1) would be a convenient precursor. In a previous study we isolated 1 in large quantities from the aerial part of *S. mellifera* Greene (Lamiaceae) which grows abundantly in California below 2000 feet.

The cyclization reaction of 1 to obtain 12,16-epoxycarnosol (2), was carried out using similar conditions as in our previous work (Chart 1), but now the reaction time was 10 d and the yield of 2 after purification was higher (95.3%).

Treatment of 2 with the Fremy’s salt (potassium nitrosodisulfonate) using acetonitrile as solvent gave a mixture of two compounds, 3 (66.4%) and 4 (6.2%). When the same reaction was carried out with the addition of tricaprylylmethylammonium chloride (Aliquat 336) in dichloromethane, compound 3 was obtained as a single product in 90.2% yield (Chart 1).

The mass spectrum of 3 showed a molecular ion [M]⁺ at *m/z* 342 (C₂₉H₂₄O₃ by HR-MS). The IR spectrum showed characteristic bands of lactone (1752 cm⁻¹) and *p*-quinone (1678 cm⁻¹) groups. In the ¹H-NMR spectrum, signals for a methyl doublet (δ 1.32, *J*= 6.8 Hz) and two angular methyls (δ 0.89, 0.90) were observed. No aromatic proton was ob-
served, which confirmed the presence of a p-quinone group on the C ring. In the low field region of the spectrum, one proton doublet of doublet at $\delta$ 5.76 was assigned to H-7. The low chemical shift of the methyl doublet ($\delta$ 1.32), and the chemical shifts and multiplicities of the coupling constants of the signals at $\delta$ 3.56, 4.26, and 4.77, were characteristic of the H-15, H-16, and H-16' proton signals present in the methylfuran system in isocryptotanshinone and other tanshinones. The $^{13}$C-NMR spectrum showed signals for 20 carbon atoms, including a signal at $\delta$ 173.5 (s) assigned to the lactone group (C-20) and signals at $\delta$ 69.6 (d), 80.6 (t), 158.7 (s), 176.6 (s), and 179.2 (s) assignable to five carbons bearing oxygens. The NOE effect observed in a ROESY experiment between H-7 and the three-proton doublet corresponding to the methyl group on C-15 confirmed the relative configuration of this carbon with the methyl group in the alpha position. This is in accordance with the absolute configuration of C-15 in the starting material 2. All these data confirm the structure of 11,14-dioxo-12,16-epoxy-8,12,15(16)-abietatrien-20,7$\beta$-olide for 3.

The spectral and physical data of 4 were similar to those of 3, where the only differences were the presence in the $^1$H-NMR spectrum of a three-proton singlet at $\delta$ 2.21 attributable to a methyl group on C-15 and a proton singlet ($\delta$ 7.45) assignable to H-16. These data confirm the presence of a methylfuran system and is in accordance with the structure of 11,14-dioxo-12,16-epoxy-8,12,15(16)-abietatrien-20,7$\beta$-olide for 4.

Treatment of 2 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in benzene at room temperature (Chart 1) gave a compound that was identified as 5. Compound 5, C$_{20}$H$_{22}$O$_4$ by HR-MS, had IR bands for phenol (3389 cm$^{-1}$) and lactone (1729 cm$^{-1}$) groups. The $^1$H-NMR spectrum showed signals for two angular methyls ($\delta$ 0.87, 0.94), a methyl group at $\delta$ 2.21 as a singlet, a benzilic proton H-7 at $\delta$ 5.52 as a doublet of doublet, and two aromatic proton singlets at $\delta$ 6.97 and 7.40 assigned to H-14 and H-16, respectively. The $^{13}$C-NMR spectrum showed the presence of 20 carbon atoms in the molecule, among which that resonating at $\delta$ 175.7 (s) was assignable to the carbonyl of the lactone group (C-20) and signals at $\delta$ 78.0 (d), 138.7 (s), 142.0 (d), and 144.5 (s) were assignable to four carbons bearing oxygens. All these data are in accordance with the structure of 15,16-didehydro-12,16-epoxycarnosol for 5.

On the basis of the above results, we thought that 3 could be aromatized to 4 using the same reaction conditions, but no reaction product was detected. However, when 3 was treated with pyridine in benzene under reflux conditions$^{44}$ (Chart 1), we obtained three products: the expected 11,14-dioxo-12,16-epoxy-8,12,15(16)-abietatrien-20,7$\beta$-olide (4) (20.2%), the
known compound isotanshinone II (6) (12.7%), and the new product (7) (7.1%), which was identified as 1-oxoisotanshinone II as follows. The high-resolution mass spectrum of 7 showed the molecular formula C_{20}H_{26}O_{5} with a molecular ion at m/z 308.1070. The IR spectrum had bands for benzilic ketone (1702 cm⁻¹) and p-quinone (1673 cm⁻¹) groups. Its 1H-NMR spectrum was similar to that of the known compound isotanshinone II (6) with the remarkable exception of the appearance of two triplets at δ 2.05 and 3.00, assignable to H-3 and H-2 protons, respectively. These data together with its mass spectral data are in accordance with a ketone group on the C-1 carbon. All the above data are in agreement with the structure of 1-oxoisotanshinone II for 7.

When 3 was treated with a stronger base, the result was a product of decarboxylation along with D-ring opening, and it was identified as the known compound (2R,5R)-neocryptotanshinone (8) (Chart 1).

Danheiser et al. (46) described a cyclization reaction of 8 with H_{2}SO_{4} in ethanol to obtain cryptotanshinone, which had the D-ring on C-14. On the same lines, we treated 3 with aqueous potassium hydroxide in THF (Chart 1), obtaining a compound that was identified from its spectroscopic data as 16-hydroxycolumbaridione (9) (Chart 1). The high-resolution mass spectrum of 9 showed the molecular formula C_{20}H_{26}O_{5} with a molecular ion at m/z 362.1766. The IR spectrum had bands for phenolic (3352 cm⁻¹), γ-lactone (1748 cm⁻¹), and quinone (1635 cm⁻¹) groups. Its 1H-NMR spectrum was similar to that of columbaridione (47) a diterpenoquinone isolated by us from Salvia columbariae, although the most remarkable difference was the appearance of a monohydroxylated isopropyl group as one of the aromatic ring substituents (3H, d, J=7.0 Hz at δ 1.21; 1H, sext, J=6.8 Hz at δ 3.30; and 2H, m at δ 3.83). The presence of two hydroxyl groups in the molecule was confirmed by the acetylation reaction of 9, using acetylchloride and lutidine in dichloromethane at room temperature, yielding the diacetylated derivative 12,16-diacetoxycolumbaridione (10). These data are in agreement with the structure of 16-hydroxy-columbaridione for 9.

When we treated 9 under the same conditions as used by Danheiser et al. (46) no cyclization reaction product was detected.

In an attempt to obtain the D-ring on C-14 present in the skeleton of some tanshinones, we treated 1 with tert-butyl-dimethylsilylethylechloride to give the C-16 protected derivative 11, which was treated with Ag_{2}O in diethyl ether to yield the o-quinone 12 (Chart 2). Compound 12, C_{26}H_{38}O_{5}Si by HR-MS, had IR bands for lactone (1753 cm⁻¹) and o-quinone (1665 cm⁻¹) groups. The 1H-NMR spectrum showed signals for the TBDMS group (δ -0.016, 0.014, 0.85), two angular methyls (δ 0.89), and a benzilic proton H-7 (δ 5.17). The absence of an aromatic proton, the appearance of a monohydroxylated protected isopropyl group as an aromatic ring substituent. At low field (δ 6.77) one proton singlet was assigned to H-14. All these data are in accordance with the structure of 16-(tert-butyl-dimethylsiloxy)-11,12-O-didehydrocarnosol for 12.

The deprotection reaction of 12 using Dowex in acetone gave a mixture of 1 (92.4%) and 3 (6.6%); however, when methanol was used as solvent, a new product 13 was obtained (Chart 2). In both cases no reaction product with the D-ring on C-14 was detected. The structure of the new product 13 was established to be 14-methoxy-12,16-epoxy-carnosol as follows. The low-resolution mass spectrum of 13 showed a molecular ion [M]⁺ at m/z 358 (C_{26}H_{30}O_{5} by HR-MS). The IR spectrum showed characteristic bands for phenol (3500 cm⁻¹) and lactone (1746 cm⁻¹) groups. Its 1H-NMR spectrum was similar to that of 12,16-epoxy-carnosol (40) although the most remarkable differences were the absence of an aromatic proton, the appearance of a methoxy group on an aromatic ring, and the low chemical shifts of the H-7 and H-15 protons. The above data are all in accordance with the structure of 14-methoxy-12,16-epoxy-carnosol for 13.

**Experimental**

**General Experimental Procedures** The NMR spectra were recorded on Bruker Avance 300-MHz and Bruker Avance 400-MHz spectrometers in CDCl₃. Chemical shifts are given in ppm with TMS as the internal standard. IR spectra were obtained on a Bruker IFS 28/55 (FTIR) spectrometer and UV spectra on a JASCO V-560. Low-resolution mass spectra were recorded on a VG Micromass ZAB-2F and high-resolution mass spectra on a VG Micromass ZAB-2F at 70 eV. Merck silica gel (0.063—0.200) was used for column chromatography. Analytical thin-layer chromatography (TLC) and preparative TLC were carried out on precoated Macherey-Nagel plates.
Isolation of 16-Hydroxycurcumin (1)  
*S. mellea* Greene was collected on the hillsides of Bluebird Canyon Road, Laguna Beach, California, in August 1988 and a voucher specimen is on file at the Museum of Systematic Biology, University of California, Irvine. The dried, ground stems and leaves (934 g) were extracted with distilled acetone at room temperature and the solvent eliminated under reduced pressure at 40 °C, giving an extract (138 g) that was subjected to flash chromatography on silica gel with mixtures of 

hexane/ethyl acetate of increasing polarity. The fraction eluted with 

hexane/ethyl acetate (1:1) contained 1. Repeated chromatography on Sephadex LH-20 and silica gel of this fraction gave 1 (520 mg).

**Treatment of 16-Hydroxycurcumin (1) with N-Bromosuccinimide**  
To a solution of N-bromosuccinimide (123 mg, 0.691 mmol) in dry dichromethane (15 ml) was added triphenylphosphine (179.6 mg, 0.684 mmol), and the mixture was stirred at room temperature for 5 min. Pyridine (0.04 ml, 0.496 mmol) was then added dropwise to the solution, followed by the addition of 16-hydroxycurcumin (1) (78.8 mg, 0.228 mmol). The reaction mixture was stirred at room temperature for 10 h and then poured into a saturated aqueous sodium bicarbonate solution. The product was extracted with ethyl acetate, washed with water and brine, and dried over anhydrous sodium sulfate. The crude product was chromatographed over silica gel using dichloromethane/acetone (99:1) as eluent to give 12,16-epoxycurcumin (2) (71.2 mg, 95.3%).

**Treatment of 2 with Fremy’s Salt**  
Method (A): Fremy’s salt (potassium nitrate disulfonate, 0.580 mmol) was dissolved in a buffer solution of Na2HPO4 (10.8 ml, pH 7), under nitrogen and in the light of 12,16-Epoxycurcumin (2) (49.7 mg, 0.152 mmol) was dissolved in acetonitrile (10.8 ml) and added to the above solution. The mixture was stirred at room temperature for 5 h and then was extracted with ethyl acetate, washed with brine, and the organic layers dried over anhydrous Na2SO4. The solvent was eliminated under reduced pressure, and the crude product was purified by preparative TLC using dichloromethane/acetone (99:1) as eluent to yield two products, 11,14-dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) (34 mg, 66.4%) and 11,14-dioxy-12,16-epoxy-8,12,15(16)-abietinatien-20,7-β-olide (4) (3.2 mg, 6.2%).

Method (B): To a buffer solution of Na2HPO4 (30 ml), pH 7, was added a solution of Aliquat 336 (0.1 ml, 0.219 mmol) and 12,16-Epoxycurcumin (2) (58.4 mg, 0.178 mmol) in dichloromethane (8 ml) and Fremy’s salt (135 mg, 0.504 mmol). The reaction mixture was stirred at room temperature for 2 h and then extracted with dichloromethane, washed with brine, and dried over anhydrous Na2SO4. The solvent was eliminated under reduced pressure, and the crude product was purified by silica gel column chromatography using dichloromethane as eluent to obtain 11,14-dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) (55.0 mg, 90.2%).

11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3): Obtained as an amorphous solid. UV (EtOH) λmax (log ε) 247 (4.02), 253 (4.33), 256 (4.34), 303 (5.39), 361 (4.02) nm. IR (film) νmax 3030, 2955, 2930, 1780, 1667, 1583, 1464, 1445, 1420, 1387, 1328, 1290, 1238, 1188, 1109, 1093, 1038, 756 cm⁻¹; 1H-NMR (300 MHz) δ 1.25 (3H, s, Me-19), 1.56 (9H, s, Me-18), 2.05 (2H, t, J = 7.3 Hz, H-3, 3.73 (3H, s, Me-17), 3.04 (2H, t, J = 7.3 Hz, H-2), 3.50 (2H, t, J = 7.3 Hz, H-2), 7.66 (1H, d, J = 8.3 Hz, H-7), 7.80 (2H, d, J = 8.2 Hz, H-6), 8.06 (1H, d, J = 8.3 Hz, H-6); EI-MS m/z 304/303 [M⁺] (25), 289 (12), 265 (42), 252 (100), 165 (8), 115 (87), 55 (5); [1]b25 = 4.00° (c = 0.05, CHCl₃); HR-ESI-MS m/z 304.07074 (Caled for C₁₉H₁₆O₄, 304.07215).  

Treatment of 11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) with BuOK in Dimethylsulfoxide  
3 (4.8 mg, 0.014 mmol) was dissolved in 2 ml of DMSO and then BuOK (8.5 mg, 0.076 mmol) was added and heated at 100 °C for 4 h. Then the reaction mixture was cooled to room temperature, and the crude product was purified by preparative TLC using dichloromethane/acetone (9:5:5) as eluent to give an amorphous solid, mp 208 °C; UV (EtOH) λmax (log ε) 227 (4.02), 253 (4.33), 256 (4.34), 303 (5.39), 361 (4.02) nm. IR (film) νmax 3030, 2955, 2930, 1780, 1667, 1583, 1464, 1445, 1420, 1387, 1328, 1290, 1238, 1188, 1109, 1093, 1038, 756 cm⁻¹; 1H-NMR (300 MHz) δ 1.25 (3H, s, Me-19), 1.56 (9H, s, Me-18), 2.05 (2H, t, J = 7.3 Hz, H-3, 3.73 (3H, s, Me-17), 3.04 (2H, t, J = 7.3 Hz, H-2), 3.39 (2H, t, J = 7.3 Hz, H-2), 7.66 (1H, d, J = 8.3 Hz, H-7), 7.80 (2H, d, J = 8.2 Hz, H-6), 8.06 (1H, d, J = 8.3 Hz, H-6); EI-MS m/z 304/303 [M⁺] (25), 289 (11), 265 (41), 252 (100), 165 (8), 115 (87), 55 (5); [1]bb = 4.00° (c = 0.05, CHCl₃); HR-ESI-MS m/z 304.07074 (Caled for C₁₉H₁₆O₄, 304.07215).  

**11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) with KOH in THF**  
11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) (53.4 mg, 0.157 mmol) was dissolved with THF (3 ml) and was cooled at 0 °C. Then an aqueous solution of 1% KOH (3 ml) was added and after 30 min the reaction mixture was acidified with 5% HCl, extracted with ethyl acetate, washed with brine, and dried over anhydrous Na2SO4. The solvent was eliminated on a rotary evaporator, and the crude product was purified by preparative TLC using dichloromethane/acetone (9:5:5) as eluent to give (+)-neocryptotanshinone (8) (2.1 mg, 47.7%).

**11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) with Butanol**  
11,14-Dioxy-12,16-epoxy-8,12-abietadien-20,7-β-olide (3) (25.0 mg, 0.080 mmol) was dissolved in 1 ml of butanol and then the solution was evaporated at 0 °C. Then an aqueous solution of 1% KOH (1 ml) was added and the reaction mixture was acidified with 5% HCl, extracted with ethyl acetate, washed with brine, and dried over anhydrous Na2SO4. The solvent was eliminated on a rotary evaporator, and the crude product was purified by preparative TLC using dichloromethane/acetone (9:5:5) as eluent to give (+)-neocryptotanshinone (8) (2.1 mg, 47.7%).
Acetylation of 16-Hydroxycurcumarinic acid (9) Compound 9 (18.1 mg, 0.0503 mmol) was dissolved in 3 ml of CHCl₃ and cooled at 0°C. Then acetyl chloride (0.1 ml, 0.861 mmol) and lutidine (0.1 ml, 0.0806 mmol) were added. After 45 min, the reaction mixture was acidified with aqueous 5% HCl, extracted with CHCl₃, and the organic layers were washed with brine and dried over anhydrous Na₂SO₄. The solvent was evaporated on a rotary evaporator, and the crude extract was purified by preparative TLC using n-hexane/ethyl acetate (9:1) as eluent to give 12,16-diacetylcarnosol (10) (6.5 mg, 29.1%).

12,16-Diacetylcarnosol (10): Obtained as an amorphous solid. UV (EtOH) λmax (log ε) 256 (2.97) nm; IR (film) νmax 2954, 1745, 1640, 1460, 1372, 1197, 1091, 918, 759 cm⁻¹; 1H-NMR (300 MHz) δ 0.88 (3H, s, Me-19), 0.90 (3H, s, Me-18), 1.22 (3H, d, J = 7.0 Hz, Me-17), 1.98 (3H, s, CH₃OAc), 2.54 (3H, s, ArOAc), 2.72 (1H, br d, J = 14.2 Hz, H-1β), 3.34 (1H, sext, J = 7.1 Hz, H-15), 4.24 (2H, m, H-16), 5.78 (1H, t, J = 7.0 Hz, H-7); 13C-NMR (75 MHz) δ 15.2 (q, C-17), 18.2 (t, C-2), 19.4 (q, C-18), 20.2 (q, CH₃OAc), 27.7 (t, C-1), 29.7 (t, C-6), 30.9 (C-15), 32.1 (q, C-19), 34.7 (s, C-4), 40.4 (t, C-3), 44.7 (d, C-5), 49.3 (s, C-10), 66.1 (t, C-16), 69.8 (d, C-7), 134.7 (s, C-13), 141.9 (s, C-8), 145.6 (s, C-9), 150.1 (s, C-12), 168.0 (s, CH₂OAc, C-5), 20.8 (q, ArOAc), 173.2 (s, C-20), 176.0 (s, C-11), 181.5 (s, C-14); EI-MS m/z 444 [M⁺] (3), 406 (2), 358 (38), 314 (90), 290 (100), 284 (17), 245 (11), 229 (39), 215 (8), 187 (29), 173 (7), 69 (15); HR-ESI-MS m/z 444.1785 (Calcd for C₂₆H₃₈O₅Si, 444.1784).

Protection Reaction of 16-Hydroxycarnosol (1) as tert-Butyldimethyl Ether To a solution of 16-hydroxycarnosol (129.1 mg, 0.8841 mmol) in dry CH₂Cl₂ (10 ml) was added imidazole (6.9 mg, 0.10 mmol), tert-butyldimethylsilane (29.3 mg, quantitative yield) and Dowex 50WX8-200 (122 mg, 8-fold the weight of the product) in dry acetone (5 ml) under nitrogen. The reaction mixture was stirred under nitrogen for 2 d and then filtered. The filtrate was removed under reduced pressure. The crude product was chromatographed over silica gel with dichloromethane/acetone (99 : 1) and increasing polarity to give 16-hydroxycarnosol (11) (34.5 mg, 89.1%).

16-(tert-Butyldimethylsilyloxy)-11,12-dideoxycurcumarinic acid (11) (96.7 mg, 0.2626 mmol) was dissolved in 5 ml of MeOH was added Dowex 50WX8-200 (11.2 mg, 60-fold the weight of the product) in dry acetone (8 ml) and then added to a mixture of Dowex 50WX8-200 (136.4 mg, 11-fold the weight of the product) in dry acetone (5 ml) under nitrogen. The reaction mixture was stirred under nitrogen for 2 d and then filtered. The solvent of the filtrate was removed under reduced pressure. The crude product was chromatographed over silica gel with dichloromethane/methanol/acetone (99 : 1 : 1). The crude product was purified using silica gel column chromatography with CH₂Cl₂ as eluent to give 14-methoxy-12,16-diepoxycarnosol (13) (8.5 mg, 92.4%) and 11,14-dioxo-12,16-epoxy-8,12-abietadien-20,7-dione (3) (0.6 mg, 6.6%).

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References