Oxidation, Reduction, and Methylation of Carnosic Acid by *Nocardia*

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Preparative-scale incubations with *Nocardia* sp. NRRL 5646 were conducted to produce new derivatives of the abietane diterpene chemoprotectant and antioxidant carnosic acid (**1**). Reductive biotransformation of the C-20 carboxylic acid functional group followed by biological methylation at the C-11 phenol afforded **4**. Oxidative cyclization of **1** to carnosol **5** followed by dihydroxylation at the isopropyl moiety afforded **6**. Metabolites **4** and **6** are new carnosic acid derivatives whose structures were confirmed by mass spectrometry and NMR spectroscopic analysis. The radical quenching properties of **4**−**6** using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging assay showed activities similar to that of mixed tocopherols and carnosic acid.

Diterpenes play diverse functional roles in plants, acting as hormones (gibberellins), photosynthetic pigments (phytol), and regulators of wound-induced responses (abietic acid).[1] Carnosic acid (**1**) is an abundant abietane diterpene found in the widely used Lamiaceae herbs rosemary and sage.[2,3] Carnosic acid is a precursor of many other related diterpenes[4] including carnosol (**5**), and both of these compounds are powerful antioxidants.[3,5] The *o*-diphenol (catechol) structure of **1** is responsible for its ability to inhibit lipid peroxidation and superoxide generation in isolated chloroplasts and microsomes versus chemically induced oxidative stresses.[6]

Masuda et al.[7] suggested that hydrogen donation from the 11-position phenol to radical species, such as lipid peroxy radical, was involved in carnosic acid's antioxidant activity. Antioxidant properties of **1** may explain the modulation of drug metabolizing enzymes involved in carcinogen activation and detoxification.[8] Carnosic acid and carnosol (**5**) inhibit cytochrome P450 activation of carcinogens in human cells in vitro[9] and enhance the activities of conjugating enzymes involved in carcinogen detoxification pathways in vivo.[10] Moreover, carnosic acid enhances gene expression consistent with 1α,25-dihydroxyvitamin D_3, all-*trans*-retinoic acid, or 12-*O*-tetradecanoylphorbol-13-acetate induced monocytic differentiation of HL-60-G cells, resulting in decreased cell proliferation and blocking cell cycle transition from G_1 to S phase.[11]

In this study we examined microbial transformations of carnosic acid as a means of preparing novel derivatives. We report here the formation of three major metabolites of **1** formed by carboxylic acid reduction, phenol methylation, and methyl group hydroxylation.

Results and Discussion

Of 49 microorganisms screened for their abilities to catalyze the bioconversion of carnosic acid (**1**), *Nocardia* sp. NRRL 5646 reproducibly formed three major metabolites of **1** in good yield. Of these, two were previously unknown carnosic acid derivatives (**4** and **6**), and one was the known natural product carnosol (**5**) (Figure 1). None of the observed metabolites were formed in control cultures or in media containing no microorganisms but incubated under the same conditions. Because of the antioxidant characteristics of **1** embodied in the catechol moiety, we were surprised that **1** did not spontaneously form **5**, or 7α-hydroxycarnosic acid by autoxidation. The lack of 7α-hydroxycarnosic acid in incubation mixtures containing **5** rules out autoxidation of **1** and introduction of oxygen from water at position 7.

Metabolites **4**−**6** were obtained from preparative-scale incubations of **1** with *Nocardia* sp. NRRL 5646 after 72 h of incubation in 10.5%, 38%, and 8% yields, respectively. Following solvent extraction and column chromatographic purification, samples of metabolites were subjected to spectral analysis.

Metabolite **4** gave $C_{21}H_{33}O_3$ (*m*/*z* 333, M + H^+) by HRESIMS, which indicated the addition of one carbon, the removal of one oxygen, and the reduction of an unsaturated center versus **1**. As with **1**, the ^{1}H NMR spectrum of **4** showed four methyl group signals at δ 0.86 (3H, s, Me-18α), 1.12 (3H, s, Me-19β), 1.16 (3H, d, J = 7.0 Hz, Me-16), and 1.19 (3H, d, J = 7.0 Hz, Me-17), confirming the presence of geminal 18,19-methyl groups and the isopropyl moiety attached at position 13. The *m*/*z* 288 fragment in the MS spectrum represented the loss of the isopropyl group from the molecular ion. Signals for all ring protons and carbons were confirmed by DQF-COSY, HMQC, and HMBC spectra. The major differences in **4** versus **1** were in the absence of the C-20 carboxyl group signal, the presence of two double doublets as an AB system at δ 3.87 (1H, dd, J = 2.7, 11.7 Hz, H-20A) and 4.12 (1H, dd, J = 6.3, 11.7 Hz, H-20B), and a 3H singlet at δ 3.86 for a methoxyl group. The hydroxy-methylene group was correlated with a carbon signal at δ 69.22 in the HMQC spectrum and showed long-range coupling with H-1α by DQF-COSY to suggest that the carboxylic acid group on C-10 of **1** was reduced to an alcohol in **4** by *Nocardia* sp. The methoxyl group was correlated with the carbon resonance at δ 152.30 (C-12) by HMBC. Irradiation of **4** at δ 3.86 (12-*O*-methyl protons) caused NOE enhancement in the signal of H-15 at δ 3.09 (8%) and a minor effect on the signals of the isopropyl methyl groups at δ 1.16 and 1.19 (2% each). Thus, the methoxyl and the isopropyl groups were *ortho* to each other.

The significant HMBC cross-peaks observed from the hydroxy-methylene protons at δ 69.22 with the methylene

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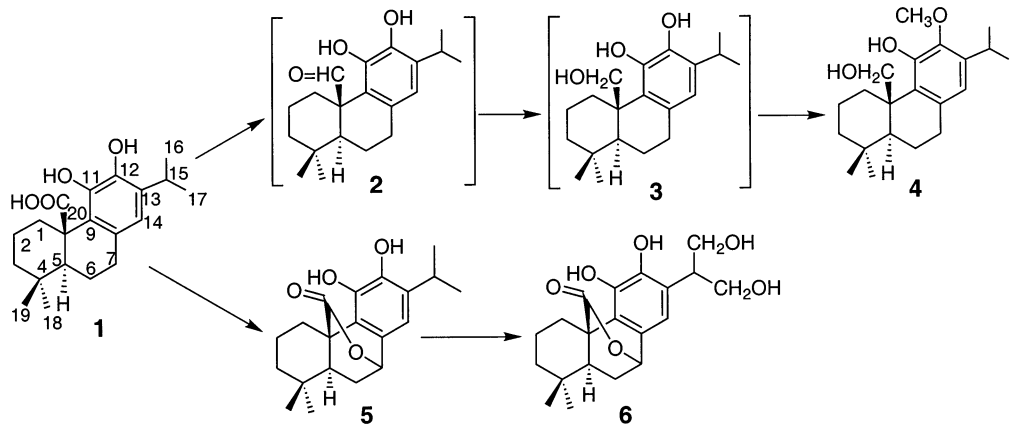

Figure 1. Stuctures of carnosic acid (**1**), metabolites formed by *Nocardia* sp., NRRL 5646, and proposed pathways for their formation.

carbon at δ 38.35 (C-1), the methine carbon at δ 52.18 (C-5), and quaternary carbons at δ 128.14 (C-9) and 42.70 (C-10) were also consistent with a C-20 alcohol. Therefore, the structure of new metabolite **4** was 11,20-dihydroxy-12-methoxyabiet-8,11,13-triene.

Metabolite **5** gave *m/z* 331 $[M + H]^+$ for $C_{20}H_{27}O_4$ by ESIMS, which indicated a structure with two less hydrogens versus **1**, which could be accounted for by a structure like carnosol. Spectral data showed that **5** was the same as carnosol[12] or 11,12-dihydroxy-7,10-(epoxymethano)abiet-8,11,13-triene-20-one.

Metabolite **6** gave *m/z* 363 $[M + H]^+$ for $C_{20}H_{27}O_6$ by HREIMS, indicating a structure like **5** plus two additional hydroxyl groups. The ^1H and ^{13}C NMR spectra of **6** showed signals with chemical shifts, multiplicities, and coupling constant similar to those of **5** except for the replacement of the signals of the isopropyl moiety of **5** with those of a dihydroxylated isopropyl moiety. That is, the ^1H and ^{13}C NMR spectra of **6** showed additional signals of two oxygenated methylene groups at δ 3.86 (1H, dd, J = 2.6, 10.8 Hz, H-16A), 4.09 (1H, dd, J = 6.3, 10.8 Hz, H-16B), 3.93 (1H, dd, J = 2.8, 10.8 Hz, H-17A), and 4.12 (1H, dd, J = 6.5, 10.8 Hz, H-17B). The C-15 methine proton at δ 3.39 (1H, quintet, J = 7.0 Hz, H-15) was shifted downfield relative to that of **1**. Two singlet protons at δ 6.37 and 6.85 that disappeared after addition of D_2O were assigned to the phenolic hydroxy groups.

HMQC and HMBC spectra of **6**, as well as the characteristic fragment ion at *m/z* 286 consistent with loss of a dihydroxylated isopropyl moiety in the ESIMS, confirmed the location of the two new hydroxyl groups at positions 16 and 17. Therefore, **6** was identified as the new metabolite 11,12,16,17-tetrahydroxy-7,10-(epoxymethano)abiet-8,-11,13-triene-20-one.

To evaluate the three carnosic acid metabolites **4**–**6** for antioxidant potential, their DPPH free-radical-scavenging activities were compared with those of carnosic acid and other antioxidants including mixed tocopherols, ascorbic acid, pyrogallol, and propyl gallate (Table 1). Among three biotransformation products, **5** exhibited the lowest activity in DPPH quenching (IC_{50} = 37.9 μg/mL). Metabolites **4** (IC_{50} = 27.7 μg/mL) and **6** (IC_{50} = 23.9 μg/mL) showed slightly less DPPH activity than carnosic acid (IC_{50} = 18.7 μg/mL) but slightly more than mixed tocopherols (IC_{50} = 28.4 μg/mL). These findings emphasize that the previously unknown metabolites **4** and **6** could be better antioxidants than tocopherols and carnosic acid in radical-scavenging processes.

Nocardia sp. NRRL 5646 contains an astonishing array of interesting and useful enzymes, many of which are similar to those found in mammals. A soluble form of nitric oxide synthase (NOS_{NOC}),[13] carboxylic acid reductase,[14] aryl aldehyde oxidoreductase,[15] aldehyde reductase,[16] guanylate cyclase,[17] and ornithine transcarbamoylase[18] have all been characterized in this organism. Activities for *Nocardia* cyclohydrolase I, sepiapterin reductase, vinylphenol hydratase,[19] hydroxyethylphenol oxidoreductase,[19] and vanillate decarboxylase are all under investigation in our laboratory.

Table 1. Activities of Metabolites **4**–**6** in the DPPH Free-Radical-Scavenging Assay

sample	IC_{50} (μg/mL)[a]	IC_{50} (μM)[a]
1	18.7	53.3
4	27.7	83.4
5	37.9	114
6	23.9	66.0
mixed tocopherols[b]	28.4	
ascorbic acid	11.8	66.8
pyrogallol	4.8	37.8
propyl gallate	9.8	46.1

Conversions of carnosic acid (**1**) to metabolites **4**, **5**, and **6** by *Nocardia* provides a reproducible means of affording known (**5**) and new derivatives of **1**. Microbial reduction of the carboxylic acid moiety of **1** adds a structurally complex terpene natural product to the wide list of substrates that are efficiently reduced by the carboxylic acid reductase and aldehyde reductase enzyme systems of *Nocardia*.[15,20,21] The *Nocardia* carboxylic acid reductase is a unique enzyme that binds carboxylic acids, ATP, and NADPH.[20] The first intermediate produced is a carbonyl-activated acyl-adenylate[20] derivative that is reduced by hydride delivery from NADPH to afford aldehydes. In whole cell *Nocardia* cultures, a separate NADPH-dependent, alcohol oxidoreductase reduces aldehydes to the corresponding alcohols (**3**). The methoxyl group at position 12 in **6** is likely introduced by means of an *S*-adenosylmethionine-dependent catechol-*O*-methyl transferase (COMT) system similar to that recently characterized by us in *Streptomyces griseus*.[22] The conversion of carnosic acid (**1**) to carnosol (**5**) likely involves enzymatic oxidation of **1** to a quinoid intermediate followed by intramolecular Michael addition of the carboxylate anion at position 7. Subsequent hydroxylations of **5** to **6** are catalyzed by unknown enzymes.

This work underlines the value of microbial transformations as a powerful biocatalytic means of preparing novel derivatives of polyfunctional natural products such as carnosic acid. Reactions observed were highly regiospecific and functional group specific, and they occurred under the mildest of reaction conditions without the need for blocking groups typically required in synthetic chemistry. The facile

biocatalytic reduction of a hindered carboxylic acid moiety by whole *Nocardia* cells illustrates the advantages and properties of microbial transformations very well in a reaction that is difficult to achieve using synthetic chemical methods. In synthesizing carnosol as a metabolite of carnosic acid, the bacterium *Nocardia* demonstrated its ability to mimic plant biochemistry in affording a known plant product.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra (cm^{-1}) were obtained using a Nicolet 205 FT-IR spectrometer connected to a Hewlett-Packard ColorPro plotter. High-resolution electrospray ionization mass spectra (HRES-IMS) were taken on a VG-ZAB-HF reversed-geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.).

NMR spectra were obtained in acetone using TMS as the internal standard, with chemical shifts expressed in δ and coupling constants (J) in Hz. Routine ^1H and ^{13}C NMR spectra were obtained with a Bruker NMR 400 (Bruker Instruments, Billerica, MA), operating at 400 MHz (^1H) and 100 MHz (^{13}C). DQF-COSY, NOESY, HMQC, and HMBC NMR experiments were carried out using a Bruker AMX-600 high-field spectrometer equipped with an IBM Aspect-2000 processor and with VNMR version 4.1 software. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated Si gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying developed plates with 1% vanillin/H_2SO_4, followed by heating at 100 °C for 3 min. TLC plates were developed with solvent system A (EtOAc/hexane, 1:1, v/v) or B ($CHCl_3$/MeOH, 8.5:1.5, v/v).

Isolation of Carnosic Acid (1). Deodorized rosemary extract (*Rosmarinus officinalis* L., leaves, 450 g) obtained through an extraction with tetrafluoroethane/acetone/methanol, 8:1:1, w/w/w) was provided as a dried residue by Kemin Foods, Inc., Des Moines, IA. The residue was exhaustively extracted at room temperature with $CHCl_3$ (3 × 5 L). The combined $CHCl_3$ extracts were concentrated in vacuo at 30 °C to a brown residue (388 g), which was fractionated by Si gel flash column chromatography (7.5 × 110 cm) using, in sequence, *n*-hexane/EtOAc (95:5 → 30:70). Eleven fractions were combined based on TLC using solvent systems A and B. Fractions containing **1** were rechromatographed by flash column chromatography over Sepralyte C_{18} using a MeOH/H_2O gradient solvent system (40 → 70%), followed by a Sephadex LH-20 (25−150 μm, Pharmacia Fine Chemical Co.) column eluted with MeOH/$CHCl_3$ (1:1) to afford carnosic acid (2.14 g) as a yellowish powder. UV, IR, ^1H and ^{13}C NMR, and ESIMS data for $[M + H]^+$ ion were in good agreement with reported data for carnosic acid (**1**) ($C_{20}H_{29}O_4$ $[M + H]^+$, 333).[2,23]

Microorganism. *Nocardia* sp. NRRL 5646 is maintained in the University of Iowa, College of Pharmacy culture collection on slants of Sabouraud-dextrose agar or sporulation agar (ATCC medium #5).[24]

Analytical-Scale Biotransformation. A two-stage fermentation protocol was used for analytical and preparative-scale formation of carnosic acid (**1**) metabolites. The medium contained 0.5% (w/v) soybean meal, 0.5% yeast extract, 0.5% NaCl, 0.5% K_2HPO_4, and 2% dextrose per 1 L of distilled water, adjusted to pH 7.0 with 6 N HCl, and autoclaved at 121 °C for 15 min. Analytical incubations were conducted in 25 mL of sterile medium held in 125 mL stainless steel-capped Delong culture flasks that were incubated with shaking at 250 rpm at 28 °C on a New Brunswick Scientific, Innova 5000 Gyrotory three-tier shaker. A 10% inoculum derived from 72 h old stage I cultures was used to initiate stage II cultures, which were incubated for 24 h more before receiving 5 mg of **1** in 0.2 mL of *N*,*N*-dimethylformamide, and incubations were continued. Substrate controls consisted of sterile medium and substrate incubated under the same conditions but without microorganism. Samples of 3 mL were withdrawn for analysis at 24, 48, 72, and 144 h after substrate addition and extracted with 1 mL of EtOAc. The organic layer was separated from aqueous medium by centrifugation at 1200*g* in a desktop centrifuge, and 30 μL samples were spotted onto TLC plates developed with solvent system A. On the basis of screening experiments, three metabolites were reproducibly formed by *Nocardia* with R_f values of 0.88 for **4**, 0.71 for **5**, and 0.54 for **6**.

Preparative Biotransformation of Carnosic Acid (1). *Nocardia* cultures were incubated as before in ten 125 mL stainless steel-capped Delong culture flasks, each containing 25 mL of medium. Carnosic acid (**1**) (100 mg) was dissolved in 1 mL of *N*,*N*-dimethylformamide and evenly distributed among the 24 h old stage II cultures. After 72 h, the contents of 10 flasks were combined and centrifuged at 10000*g* at 4 °C for 20 min. The supernatant (225 mL) was extracted with three 500 mL volumes of EtOAc. The organic layers were pooled, dried over anhydrous Na_2SO_4, filtered through sintered glass, and vacuum-concentrated to yield 93 mg of a viscous brown residue.

Isolation of Metabolites. The brown residue was subjected to Si gel flash column chromatography (1.5 × 50 cm) using in sequence hexane/EtOAC (95:5 → 75:25). Two fractions, A (22 mg) and B (53 mg), were obtained based on TLC analysis. Fractions A and B were separately resolved by reversed-phase Sepralyte C_{18} Si gel flash column chromatography (1 × 50 cm), using a MeOH/H_2O gradient solvent system (40 → 70%) under column pressures of 0.28 kg/cm^2, at a flow rate of 2 mL/min, while 3 mL fractions were collected. Final sample purifications were carried out with Sephadex LH-20 (25−150 μm, Pharmacia Fine Chemical Co.) columns eluted with MeOH/$CHCl_3$ (1:1) to afford carnosic acid metabolites **4** (10.5 mg), **5** (38 mg), and **6** (8 mg).

DPPH Radical-Scavenging Assay. The DPPH free-radical-scavenging assay is based on the abilities of compounds to quench stable DPPH free radicals.[25] Reaction mixtures (200 μL) were prepared by combining multiple concentrations of test sample in methanol (10 μL) with DPPH (Aldrich, Milwaukee, WI) in MeOH (190 μL). The final DPPH concentration was 300 μM. The reaction mixtures were incubated in 96-well microtiter plates at room temperature for 30 min.. After the reaction, reduction of the radical was then measured at 517 nm. Percent inhibition by sample treatment was determined by comparison with controls containing no test samples. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.[25]

Metabolite 4: yellowish crystals (10.5 mg); $[\alpha]^{25}_D$ +58.9° (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.30), 236 (3.59) nm; IR (KBr) ν_{max} 3375 (OH), 1595 and 1515 (aromatic ring) cm^{-1}; ^1H NMR (acetone, 600 MHz) δ 1.72 (1H, dt, J = 3.5, 13.5 Hz, H-1α), 3.11 (1H, brd, J = 13.5 Hz, H-1β), 1.58 (1H, dd, J = 6.2, 11.7 Hz, H-2α), 2.94 (1H, ddd, J = 5.5, 6.2, 11.7 Hz, H-2β), 1.54 (1H, m, H-3α), 2.09 (1H, m, H-3β), 1.50 (1H, d, J = 12.3 Hz, H-5α), 1.73 (1H, dd, J = 5.9, 13.0 Hz, H-6α) 1.92 (1H, dq, J = 4.8, 13.0 Hz, H-6β), 2.89 (2H, m, H_2-7), 6.88 (1H, s, H-14), 3.09 (1H, sept, J = 7.0 Hz, H-15), 1.16 (3H, d, J = 7.0 Hz, Me-16), 1.19 (3H, d, J = 7.0 Hz, Me-17), 0.86 (3H, s, Me-18), 1.12 (3H, s, Me-19), 3.87 (1H, dd, J = 2.7, 11.7 Hz, H-20A), 4.12 (1H, dd, J = 6.3, 11.7 Hz, H-20B), 3.86 (3H, s, OCH_3), 6.11 (1H, s, ArOH); ^{13}C NMR (acetone, 100 MHz) δ 38.35 t (C-1), 18.73 t (C-2), 36.76 t (C-3) 35.10 s (C-4), 52.18 d (C-5), 20.50 t (C-6), 34.48 t (C-7), 135.45 s (C-8), 128.14 s (C-9), 42.70 s (C-10), 149.68 s (C-11), 152.30 s (C-12), 137.94 s (C-13), 118.00 d (C-14), 27.50 d (C-15), 22.13 q (C-16), 22.76 q (C-17), 21.12 q (C-18), 26.55 q (C-19), 69.22 t (C-20), 56.80 q (OCH_3); HRESIMS *m*/*z* $[M + H]^+$ 333.2434 (calcd for $C_{21}H_{33}O_3$, 333.2429).

Metabolite 5: UV, IR, ^1H and ^{13}C NMR, and ESIMS data for $[M + H]^+$ ion in good agreement with reported data for carnosol ($C_{20}H_{27}O_4$ $[M + H]^+$, 331).[12]

Metabolite 6: yellowish amorphous powder (8 mg); $[\alpha]^{25}_D$ +67.2° (*c* 0.34, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.36), 287

(3.78) nm; IR (KBr) ν_{max} 3408 (OH), 1750 (γ-lactone), 1590 and 1518 (aromatic ring) cm^{-1}; ^1H NMR (acetone, 600 MHz) δ 2.14 (1H, dt, J = 5.1, 13.8 Hz, H-1α), 3.02 (1H, brd, J = 13.8 Hz, H-1β), 1.96 (1H, dd, J = 5.1, 12.8 Hz, H-2α), 2.64 (1H, dt, J = 5.1, 12.8 Hz, H-2β), 1.44 (1H, m, H-3α), 1.82 (1H, m, H-3β), 1.72 (1H, dd, J = 5.7, 10.9 Hz, H-5α), 2.68 (1H, ddd, J = 4.5, 5.7, 13.5 Hz, H-6α) 1.95 (1H, ddd, J = 1.7, 10.9, 13.5 Hz, H-6β), 5.43 (1H, dd, J = 1.7, 4.5 Hz, H-7), 6.82 (1H, s, H-14), 3.39 (1H, quint., J = 7.0 Hz, H-15), 3.86 (1H, dd, J = 2.6, 10.8 Hz, H-16A), 4.09 (1H, dd, J = 6.3, 10.8 Hz, H-16B), 3.93 (1H, dd, J = 2.8, 10.8 Hz, H-17A), 4.12 (1H, dd, J = 6.5, 10.8 Hz, H-17B), 1.10 (3H, s, Me-18), 0.95 (3H, s, Me-19), 6.37 (1H, s, ArOH), 6.85 (1H, s, ArOH); ^{13}C NMR (acetone, 100 MHz) δ 28.76 t (C-1), 19.62 t (C-2), 42.55 t (C-3) 36.35 s (C-4), 46.58 d (C-5), 30.15 t (C-6), 77.82 d (C-7), 136.30 s (C-8), 128.22 s (C-9), 49.50 s (C-10), 149.11 s (C-11), 150.10 s (C-12), 139.12 s (C-13), 118.15 d (C-14), 28.87 d (C-15), 69.33 t (C-16), 71.14 t (C-17), 22.10 q (C-18), 30.05 q (C-19), 176.52 s (C-20); HRES-IMS m/z $[M + H]^+$ 363.1811 (calcd for $C_{20}H_{27}O_6$, 363.1807).

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References and Notes

(1) Hanson, R. *Nat. Prod. Rep.* **1995**, *12*, 207−218.
(2) Curvelier, M. E.; Richard, H.; Berset, C. *J. Am. Oil Chem. Soc.* **1996**, *73*, 645−652.
(3) Löliger, J. In *The Use of Antioxidants In Food. In Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, 1991; Chapter 6.
(4) Luies, J. G.; Quinones, W.; Grillo, T. A.; Kishi, M. P. *Phytochemistry* **1994**, *35*, 1371−1374.
(5) Offord, E. A.; Guillot, F.; Aeschbach, R.; Löliger, J.; Pfeifer, A. M. A. *Antioxidant and Biological Properties of Rosemary Components: Implications for Food and Health, In Natural Antioxidants: Chemistry, Health Effects, and Application*; Shahidi, F., Ed.; AOCS Press: Champaign, IL, 1997; pp 88−96.
(6) Munné-Bosch, S.; Schwarz, K.; Alegre, L. *Free Rad. Res.* **1999**, *31*, S107−112.
(7) Masuda, T.; Inaba, Y.; Takeda, Y. *J. Agric. Food Chem.* **2001**, *49*, 5560−5565.
(8) Aruoma, O. I.; Halliwell, B.; Aeschbach, R.; Löliger, J. *Xenobiotica* **1992**, *22*, 257−268.
(9) Offord, E. A.; Mace, K.; Ruffieux, C.; Malone, A.; Pfeifer, A. M. *Carcinogenesis* **1995**, *16*, 2057−2062.
(10) Singletary, K. W. *Cancer Lett.* **1996**, *100*, 139−144.
(11) Danilenko, M.; Wang, X.; Studzinski, G. P. *J. Natl. Cancer Inst.* **2001**, *93*, 1224−123.
(12) Inatani, R.; Nakatani, N.; Fuwa, H.; Seto, H. *Agric. Biol. Chem.* **1982**, *46*, 1661−1666.
(13) (13) Chen, Y,; Rosazza, J. P. N. *J. Bacteriol.* **1995**, *177*, 5122−5128.
(14) Li, T.; Rosazza, J. P. N. *Appl. Environ. Microbiol.* **2000**, *66*, 684−687.
(15) Li, T.; Rosazza, J. P. N. *J. Bacteriol.* **1997**, *179*, 3482−3487.
(16) Li, T.; Rosazza, J. P. N. *J. Ind. Micrbiol. Biotech.* **2000**, *25*, 328−332.
(17) Son, J. K.; Rosazza, J. P. N. *J. Bacteriol.* **2000**, *182*, 3644−3648.
(18) Farid, I. S. Arginine Biosynthesis in *Nocardia* NRRL 5646 and iNOS Inhibition by Noformycin and its Analogs. Ph.D. Thesis, The University of Iowa, Iowa City, IA, 2001, pp 43−82.
(19) Lee, K. S.; Rosazza, J. P. N. *Can. J. Chem.* **2002** (in press).
(20) Chen, Y.; Rosazza, J. P. N. *Appl. Enivron. Microbiol.* **1994**, *60*, 1292−1296.
(21) Li, T.; Rosazza, J. P. N. *J. Biol. Chem.* **1998**, *273*, 34230−34233.
(22) Dhar, K.; Rosazza, J. N. P. *Appl. Enivron. Microbiol.* **2000**, *66*, 4877−4882.
(23) Schwarz, K.; Ternes, W. *Z. Lebensm Unters. Forsch.* **1992**, *195*, 99−103.
(24) Gherna, R.; Pienta, P.; Cote, R. *ATCC Cataloge of Bacteria and Bacteriophages*, 18th ed.; American Type Culture Collection: Rockville, MD, 1992; p 415.
(25) Lee, S. K.; Mbwambo, Z. H.; Chung, H. S.; Luyengi, L.; Gamez, E. J. C.; Mehta, R.G.; Kinghorn, A. D.; Pezzuto, J. M. *Comb. Chem. High Throughput Screen.* **1998**, *1*, 35−46.

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