JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antimicrobial Activity of the Ornamental Species *Salvia corrugata*, a Potential New Crop for Extractive Purposes

ANGELA BISIO,*,† GIOVANNI ROMUSSI,† ELEONORA RUSSO,† SERGIO CAFAGGI,† ANNA MARIA SCHITO,‡ BARBARA REPETTO,‡ AND NUNZIATINA DE TOMMASI§

Dipartimento di Chimica e Tecnologie Farmaceutiche e Alimentari, Università di Genova, Via Brigata Salerno 13, 16147 Genova, Italy, Dipartimento di Scienze Chirurgiche Specialistiche, Anestesiologia e Trapianti d'Organo, Largo R. Benzi 10, 16132 Genova, Italy, and Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Salerno, Italy

As a part of our search for biologically active compounds from cultivated *Salvia* spp. we investigated *Salvia corrugata* Vahl. The activity of two isolated icetaxane diterpene quinones, fruticuline A and demethylfruticuline A, was assessed against 46 bacterial pathogens, mostly resistant to several primary antibiotics. The MIC for all the inhibited Gram-positive pathogens tested showed a very narrow distribution and ranged from 32 to 64 mg/L, regardless of their resistance patterns to other antibiotics. Demethylfruticuline A was shown to be highly bactericidal (>3 $\log_{10}$ CFU decrease within 24 h) against *Staphylococcus aureus* and *S. epidermidis* and bacteriostatic against *Enterococcus faecalis* and *E. faecium*. Fruticuline A manifested bacteriostatic activity against all tested strains. *S. corrugata* can be viewed as an interesting source for these diterpenes, which, if well tolerated in vivo, may represent new medical agents useful for the treatment of serious infections caused by resistant Gram-positive pathogens.

KEYWORDS: ***Lamiaceae*; *Salvia corrugata*; fruticuline A; demethylfruticuline A; *Staphylococcus*; *Enterococcus*; multidrug resistant pathogens; antibacterial activity**

INTRODUCTION

The genus *Salvia* includes more than 900 species of plants distributed throughout the world, particularly in tropical and subtropical areas (*1*). The genus comprises herbaceous, suffructicous or shrubby perennial (rarely biennial or annual) species, many of which are grown for ornamental purposes. *Salvia* has been shown to possess biologically active constituents: these are generally found in the complex secretion products of these plants, whose secretory structures (glandular trichomes) are widely distributed on their aerial parts (*2*−*4*). The antioxidant, antibacterial and antifungal properties that these exudates have demonstrated against human pathogens (*6*−*9*) warrant the further study of their chemical constituents, which may prove useful in the search for biologically active compounds, particularly terpenoids (*1, 5*). The antimicrobial activity is particularly promising, in view of the pressing need for novel, valid therapeutic solutions against the increasing incidence and rapid spread throughout the world of drug-resistant Gram-negative and -positive pathogens. The threats posed by strains such as *Pseudomonas aeruginosa, Escherichia coli*, MRSA MRSE, VAN-R *E. faecium* and *E. faecalis*, which are often responsible for serious nosocomial and community-acquired infections, raise particular concerns.

In this study we focused our attention on *Salvia corrugata* Vahl., an American species belonging to the subgenus *Calosphace*, sectio *Corrugatae* (*10*) that is widely cultivated as an ornamental plant and easily grown in the Mediterranean coastal area. We found that the exudation product of the fresh aerial parts of this species possesses a clear antibacterial activity against some representative Gram-positive pathogens (one *Micrococcus lysodeicticus*, two MRSA, two VAN-R *E. faecium* and two VAN-R *E. faecalis*), whereas representative Gram-negative pathogens tested (two *E. coli* and two *Pseudomonas aeruginosa*) were not affected. Herein we report the isolation of ursolic acid (**1a**) in mixture with oleanolic acid (**1b**) and the isolation and quantification of the highly oxidized diterpene quinones fruticuline A (**2**) and demethylfruticuline A (**3**) as the active compounds of this plant exudate (**Figure 1**). Moreover, we describe for the first time a significant antibacterial activity of the two diterpene quinones **2** and **3** against various multidrug-resistant human Gram-positive pathogens.

MATERIALS AND METHODS

General Experimental Procedures. All reagents were of analytical or HPLC grade. Melting points are uncorrected and were measured on

* To whom correspondence should be addressed. Tel: +39-010-3532638. Fax: +39-010-3532684. E-mail: bisio@dictfa.unige.it.

† Dipartimento di Chimica e Tecnologie Farmaceutiche e Alimentari, Università di Genova.

‡ Dipartimento di Scienze Chirurgiche Specialistiche, Anestesiologia e Trapianti d'Organo.

§ Dipartimento di Scienze Farmaceutiche, Università di Salerno.

10.1021/jf802200x CCC: $40.75
Published on Web 10/31/2008

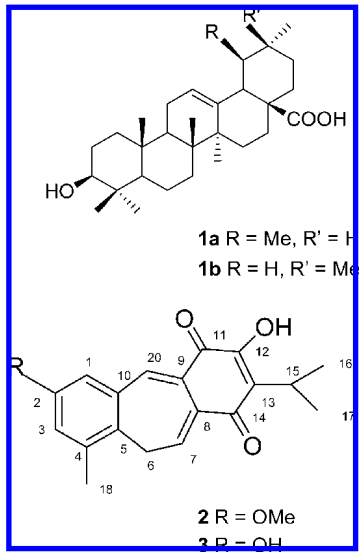

Figure 1. Structures of ursolic acid (**1a**), oleanolic acid (**1b**), fruticuline A (**2**), demethylfruticuline A (**3**).

a Tottoli melting point apparatus (Büchi, Switzerland). Sephadex LH-20 (Pharmacia, Sweden) was used for column chromatography; aluminum sheets of silica gel 60 F_{254} (0.2 mm thick, Merck, Germany) with $CHCl_3$/MeOH/HCOOH (10:0.5:0.1) as an eluent were used for TLC and the spots were detected by spraying 50% H_2SO_4 followed by heating. IR spectra were recorded on a Perkin-Elmer (USA) 1310 spectrophotometer. NMR spectra were recorded on a BRUKER DRX 600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C) in $CDCl_3$ using TMS as an internal standard. Deionized water was purified by Milli-Q plus system (Millipore, Bedford, MA, USA). Semipreparative HPLC was carried out using a Waters W600 pump (Waters Corporation, Milford, USA) equipped with a Rheodyne Delta 600 Injector (with a 100 μL loop) and a Waters 2414 refractive index detector. The reversed-phase semipreparative chromatography was performed at room temperature on a chemically bonded stationary phase, 10 μm μBondapack C18 column (7.8 × 300) mm (Waters). The elution mixture (helium-degassed) was composed of CH_3CN/H_2O 75:25. The flow rate was 2.0 mL/min. The elution time was 20 min. Analytical HPLC was performed using an HP 1090 Series II HPLC system, equipped with a UV diode array detector (Hewlett-Packard, Palo Alto, CA, USA). A 5 μm LiChrospher 100 RP-18 endcapped column (250 × 4.5) mm, (Merck, Darmstadt, Germany) was used.

Quantification of **2** and **3** in the exudate was carried out by developing and validating a reversed-phase HPLC analytical method. Volume samples of 25 μL were injected and eluted with a mobile phase (helium-degassed) composed of CH_3CN/H_2O 75:25. The flow rate was 1.0 mL/min. The acquisition wavelength used for both compounds **2** and **3** was 275 nm, one of their absorption maxima in the mobile phase. The analysis time was 20 min. The retention times of **2** and **3** were 7 and 4 min, respectively. No interfering peaks were detected at the retention times of the analytes. Quantification was achieved by using peak area versus concentration on the basis of calibration curves constructed over the concentration range (40−250) μg/mL. Suitable quantities of very pure **2** and **3** for calibration curves and antimicrobial tests were obtained by semipreparative HPLC purification. Standard solutions of **2** and **3** in acetonitrile were prepared at five different concentrations (40, 90, 140, 190, 230 μg/mL). Calibration curves were linear ($r^2 > 0.999$) with an intercept not significantly different from zero. Quantitative results, expressed as substance relative amount (% w/w) in the exudate, are reported as 95% confidence intervals.

The composition of the mixture of ursolic acid (**1a**) and oleanolic acid (**1b**) was determined using the HPLC method proposed by Claude et al., 2004 (*11*).

Plant Material. Fresh aerial parts of *Salvia corrugata* Vahl were obtained from the Istituto Sperimentale per la Floricoltura (Sanremo, Italy) and Centro Regionale di Sperimentazione ed Assistenza Agricola (Albenga, Italy). The species was identified by Dr. Gemma Bramley and a voucher specimen is deposited at Kew Herbarium (K) (Kew, Surrey, U.K.).

Extraction and Isolation of Active Compounds. For the isolation of leaf surface constituents, fresh aerial parts (2.6 kg) were immersed in CH_2Cl_2 for 20 s to obtain only the external secreted material mixed with cuticular components, thereby avoiding extraction of components inside the cell wall. After filtration, the extraction solvent was removed under reduced pressure. The exudate (25 g, 0.96% w/w of fresh plant) was chromatographed in portions of 1.0 g on Sephadex LH-20 columns (53 × 2.5 cm), using $CHCl_3$/MeOH (7:3) as an eluent. The eluate fractions (20 mL each) were combined according to TLC to obtain four main fraction groups (I−IV): group I (up to 140 mL) (waxy inactive compounds), group II (from 140 to 160 mL), group III (from 160 to 200 mL), group IV (from 200 to 260 mL). Group II was evaporated, washed with hexane and crystallized from EtOH to yield a product which was identified as a crystalline mixture of **1a** and **1b**. Group III was extracted with hot hexane. The hexane solution was evaporated and the residue was crystallized from EtOH to yield crude **2**, which was purified by recrystallization from $CHCl_3$/EtOH. Repeated recrystallizations from EtOH of the hexane insoluble residue yielded a crystalline mixture of **1a** and **1b**. Group IV was evaporated and the residue was crystallized from EtOH to yield crude **3**. All the obtained products were subsequently investigated as described below. The obtained amounts of these compounds were 4.2 g for the mixture of **1a** and **1b**, 0.76 g for **2** and 2.8 g for **3**.

Microorganisms. A total of 46 strains of three test organisms (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*), obtained from the American type Culture Collection (ATCC) and 43 clinical strains, previously isolated from different human specimens and identified according to standard procedures (*12*), were employed in this study. Of these, 5 were methicillin-susceptible *Staphylococcus aureus* (MSSA), 13 methicillin-resistant and multidrug-resistant *S. aureus* (MRSA), 4 methicillin-susceptible *S. epidermidis* (MSSE) and 5 methicillin-resistant *S. epidermidis* (MRSE), 2 vancomycin-susceptible (VAN-S) *Enterococcus faecalis*, 4 vancomycin-resistant (VAN-R) *E. faecalis*, 1VAN-S *E. faecium* and 5 VAN-R *E. faecium*; 1 *Bacillus subtilis*, 1 *Micrococcus lysodeicticus*, 3 *Escherichia coli* and 2 *Pseudomonas aeruginosa*. Our definition of multidrug-resistant organisms, in keeping with the literature (*13*) is "a strain that displays at least 3 resistance traits to chemically unrelated antibiotics".

Active Compounds. Sterile stock solutions in dimethyl sulfoxide (DMSO) of the surface exudate and of the two diterpene quinones **2** and **3** were prepared. More diluted solutions of the same compounds were obtained using the appropriate media required for the experiments.

Antimicrobial Activity of the Surface Exudate. To evaluate the antimicrobial activities of the surface exudates we followed a procedure detailed by Lorian (*14*), which entails the use of a replicator in order to spot the liquid onto the surface of an appropriately seeded agar plate. Selected Gram-positive and Gram-negative strains were grown overnight at 37 °C on Mueller Hinton plates (MH) (Biolife, Milan, Italy). After incubation, 5−7 bacterial colonies of each tested strain were suspended in sterile 0.1 M phosphate buffer (PB). The density of the bacterial suspensions was determined in a McFarland nephelometer (Dalynn Biological Inc.) and adjusted in order to obtain a final concentration of about 1.5×10^8 CFU/mL. Aliquots of 0.01 mL of the bacterial suspensions were spread on MH agar plates, and 10.0 μL of the surface exudate at several 2-fold dilutions in DMSO (ranging from 50.000 mg/L to 400 mg/L) were spotted on the surface of the plate. The plates were then incubated overnight at 37 °C, and the diameters of the resulting zones of inhibition were analyzed. Each experiment was performed in duplicate. For control purposes, pure DMSO was also spotted on the same MH plate.

Susceptibility Testing. The MICs of compounds **2** and **3** were determined following the microdilution procedure detailed by the Clinical and Laboratory Standards Institute (*13*) using cation-adjusted MH broth as a test medium. Briefly, overnight cultures of bacteria were diluted to yield a final concentration of about 5×10^5 cells/mL. Samples were then added to equivalent volumes of various concentrations of

compounds **2** and **3** distributed on a microplate and prepared from serial 2-fold dilutions, ranging from 0.015 to 256 μg/mL. After 24 h incubation at 37 °C, the lowest concentration of substances **2** or **3** that prevented a visible growth was recorded as the MIC. All MICs were obtained in duplicate.

Killing Curves. Killing curve assays for compounds **2** and **3** were performed on several representative isolates. A midlogarithmic phase culture was diluted in MH broth (10 mL) containing 4 × MIC of each substance to give a final inoculum of 1.0×10^5 CFU/mL. The same inoculum was added to plain MH broth as a growth control. Tubes were incubated at 37 °C with constant shaking for 24 h. Samples of 0.20 mL from each tube were removed at 0, 2, 4, 6, 24 h, diluted appropriately with 0.9% sodium chloride to avoid carryover of the two tested compounds, plated onto MH plates, and incubated for 24 h at 37 °C. Growth controls were run in parallel to the substance-containing test tubes. The percentage of surviving bacterial cells was determined for each sampling time by comparing colony counts with those of standard dilutions of the initial bacterial inoculum. Results are expressed as $\log_{10}$ of viable cell numbers CFU/mL of surviving bacterial cells over a 24 h period. Bactericidal effect was defined as a 3 $\log_{10}$ decrease of CFU/mL (99.9% killing) of the initial inoculum within 24 h. All time-kill curve experiments were performed in duplicate.

RESULTS AND DISCUSSION

Gram-positive bacteria such as *S. aureus*, *S.epidermidis* and *Enterococcus* species are common causes of bloodstream and other serious infections (*15*). The widespread emergence of MRSA associated with multiple resistance has deprived all beta-lactams of their original potency and has warranted the use of the more toxic glycopeptides (*16*). Recently, however, vancomycin intermediate and fully resistant VRSA have been described (*17, 18*). *S. epidermidis* is a pathogen whose importance has grown as a result of the increased use of plastic medical devices in hospitalized patients. This species has developed a lack of susceptibility to methicillin and, as a consequence, to other chemically related and unrelated drugs, with an incidence exceeding that shown by *S. aureus* (*19*). Enterococci are isolated from infections ranging from urinary tract to bloodstream infections (*15*). They are characterized by an intrinsic resistance to several antibiotics and by a rapid acquisition of resistance to primary anti-Gram-positive molecules (*20*). *P. aeruginosa* and *E. coli* are also characterized by widespread acquisition of resistance to primary antimicrobials, such as third generation cephalosporins, aminoglycosides, carbapenems and fluoroquinolones (*21*). Faced with challenging scenarios such as these, new therapeutic molecules endowed with a different mechanism of action and capable of overcoming resistance would be welcome. Our finding of bioactivity in the two compounds isolated from *Salvia corrugata* Vahl. therefore appears to be especially appealing.

Isolation and Identification of the Active Compounds. Column chromatography on Sephadex LH-20 of the surface exudate of aerial parts of *S. corrugata* yielded a crystalline mixture of **1a** and **1b** (one single spot on TLC by direct comparison with authentic samples of ursolic and oleanolic acids, and ^{13}C NMR spectrum with signals consistent with those of the two compounds (*22, 23*), the HPLC chromatogram resulting from the formation of a hydrophilic inclusion complex between γ-cyclodextrin and the isolated product (*11*) showed that the two isomers were present at a weight ratio of about 50%), fruticuline A (**2**) and demethylfruticuline A (**3**). **2** and **3** were identified by spectroscopic NMR analysis, including DEPT, TOCSY, HSQC and HMBC experiments. **2**: ^{1}H NMR δ 1.28 (d, 6, $J = 7.0$ Hz, Me 16 and 17), 2.47 (s, 3, Me-18), 3.17 (d, 2, $J = 7.6$ Hz, CH_2-6), 3.39 (m, 1, H-15), 3.82 (s, 3, OMe-2), 6.89 (d, 1, $J = 1.8$ Hz, H-1), 6.98 (t, 1, $J = 7.6$ Hz, H-7), 7.00 (d, 1, $J = 1.8$ Hz, H-3), 8.19 (s, 1, H-20); ^{13}C NMR δ 19.6 (C-16 and 17), 20.3 (C-18), 25.0 (C-15), 28.3 (C-6), 55.4 (OMe-2), 112.0 (C-1), 121.5 (C-3), 127.1 (C-5), 129.5 (C-9), 132.0 (C-13), 133.1(C-8), 134.0 (C-7), 135.6 (C-4), 136.2 (C-10), 140.9 (C-20), 154.7 (C-12), 157.4 (C-2), 183.2 (C-11), 184.1 (C-14). **3**: ^{1}H NMR: δ 1.29 (d, 6, $J = 7.2$ Hz, Me 16 and 17), 2.42 (s, 3, CH_3-18), 3.14 (d, 2, $J = 7.6$ Hz, CH_2-6), 3.40 (m, 1, H-15), 6.83 (d, 1, $J = 2.0$ Hz, H-1), 6.90 (d, 1, $J = 2.0$ Hz, H-3), 6.99 (t, 1, $J = 7.6$ Hz, H-7), 8.12 (s, 1, H-20). ^{13}C NMR: δ 19.5 (C-16 and 17), 20.1 (C-18), 25.0 (C-15), 28.3 (C-6), 114.2 (C-1), 122.0 (C-3), 127.0 (C-5), 129.6 (C-9), 132.1 (C-13), 133.1(C-8), 134.1 (C-7), 135.7 (C-4), 136.4 (C-10), 140.6 (C-20), 153.4 (C-2), 154.8 (C-12), 183.1 (C-11), 184.4 (C-14). The interpretations of proton and carbon signals of these diterpenoids were largely consistent with those of Rodriguez-Hahn et al. (*24, 25*) and Valant-Vetschera et al. (*26*). However, on the basis of DEPT, HSQC and HMBC experiments, some assignments were revised. In the ^{13}C NMR spectrum of **2** the signals at δ 134.0 and 140.9 were assigned to C-7 and C-20 and at δ 127.1, 129.5 and 132.0 to C-5, C-9 and C-13, respectively; in the ^{1}H NMR spectrum of **2** the signals at δ 6.89 and 7.00 were assigned to H-1 and H-3, respectively. In the ^{13}C NMR spectrum of **3** the signals at δ 134.1 and 140.6 were assigned to C-7 and C-20 and at δ 127.0, 129.6 and 132.1 to C-5, C-9 and C-13, respectively.

Antimicrobial Activity of the Surface Exudate. The antimicrobial activity of the surface exudate obtained from the aerial parts of *Salvia corrugata* Vahl. was evaluated by testing several 2-fold dilutions of the exudate (ranging from 50,000 mg/L to 400 mg/L) on the following selected bacterial strains: 1 *M. lysodeicticus*, 2 multidrug-resistant *S. aureus*, 2 VAN-R *E. faecium*, 2 VAN-R *E. faecalis*, 2 *E. coli* and 2 *P. aeruginosa*. The inhibition of growth of all Gram-positive pathogens was clearly detected, starting from a concentration of 1500 mg/L. By contrast, Gram-negative bacteria were totally unaffected by any concentration of the exudate (data not shown).

MICs of the Two Diterpene Quinones (2 and 3). Since ursolic acid and oleanolic acid have already shown a well-documented activity on several bacterial pathogens (*27*), only the MICs of the two not yet biologically characterized diterpene quinones **2** and **3** were determined for all the 46 strains. None of the two compounds displayed a significant activity against *P. aeruginosa* or *E. coli* (MIC values >256 mg/L), possibly implying a lack of penetration of these molecules into these Gram-negative bacteria. On the contrary, a promising antimicrobial activity was detected for **2** and **3** against all Gram-positive strains tested. Interestingly, the antibacterial potency (in terms of growth inhibition) of the two oxidized diterpene quinones was similar against all the pathogens tested. In fact, a very narrow distribution of MIC values, ranging only from 32 to 64 mg/L, was observed, regardless of species (*Staphylococcus, Enterococcus, Micrococcus, Bacillus*) or patterns of resistance to other antimicrobial drugs.

Time-Kill Curve Analysis. Twenty-four hour time-kill experiments were conducted for compounds **2** and **3** at concentrations of 4 times the MIC values on selected organisms. **Figures 2−5** summarize the results obtained. For each strain, data are plotted as the mean of two different experiments (vertical bars represent standard deviation). Fructiculine A (**2**) showed a weak bacteriostatic activity within the first 6 h against the two MRSA tested, with subsequent regrowth approaching the level of the control at 24 h (**Figure 2**). Similar patterns of action were observed with the two MRSE studied (data not shown). By contrast, demethylfructiculine A (**3**) displayed a highly signifi-

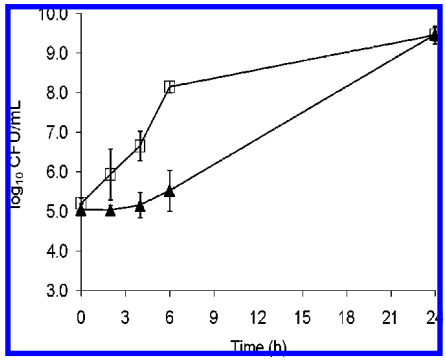

Figure 2. Effect of fructiculine A (**2**) on viable cell number of *S. aureus*. Time-kill curves against *S. aureus* (two M-RSA) in the absence (open squares) or in the presence (closed triangles) of **2** at 4 × MIC (mean of two experiments ± standard deviation).

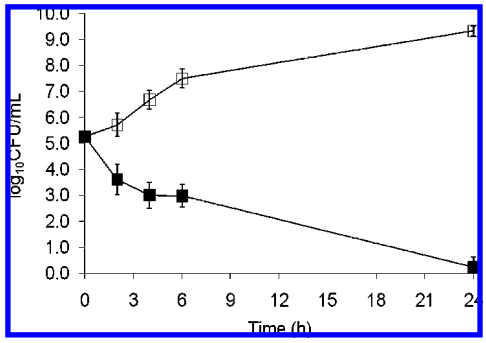

Figure 3. Effect of demethylfructiculine A (**3**) on viable cell number of *S. aureus*. Time-kill curves against *S. aureus* (three M-RSA) in the absence (open squares) or in the presence (closed squares) of **3** at 4 × MIC (mean of two experiments ± standard deviation).

cant killing activity on the three MRSA tested, with a drop in cell counts largely exceeding 3 logs, the value to be expected from a truly bactericidal agent (*20*). In fact, the number of CFU decreased from 10^5 to 10^1/mL within 24 h (**Figure 3**). More experimental data are needed to better evaluate this phenomenon. The slightly enhanced hydrophilic/lipophilic balance in compound **3**, due to the two free OH groups, appears to be insufficient to satisfactorily explain the different behavior of compound **3** against staphylococci compared to **2**. The mechanism of action underlying this effect on staphylococci will be the subject of further investigations.

On the two VAN-R *E. faecalis* tested (**Figure 4**), while compound **2** inhibited growth of the pathogens only for about 6 h, permitting thereafter full regrowth, compound **3** manifested a stronger effect with a slight killing activity (about a 1 log decrease) and lack of substantial regrowth even at very late times of observation (24 h). The same trend was apparent with the two VAN-R *E. faecium* analyzed (**Figure 5**). The lower activity profile displayed by the two new compounds against Enterococci reflects the naturally lower susceptibility of this species to several compounds in general, and that only appropriate combinations of drugs are capable of a substantial killing of these pathogens (*15*).

The activity of the two quinones described herein, bacteriostatic in the case of compound **2** on all Gram-positives and compound **3** on enterococci, and bactericidal in the case of compound **3** on staphylococci, is of special interest in view of the fact that Gram-positive organisms tend to acquire resistance traits that nullify treatment efforts. If these compounds were shown to possess different mechanisms of action compared to those of mainline antibiotics, their usefulness in overcoming

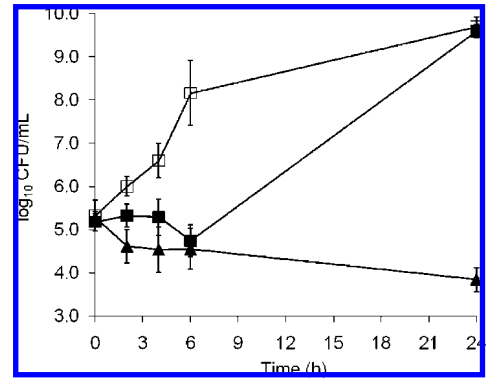

Figure 4. Effect of fructiculine A (**2**) and demethylfructiculine A (**3**) on viable cell number of *E. faecalis*. Time-kill curves against *E. faecalis* (two VAN-R) in the absence (open squares) or in the presence of **3** (closed triangles) at 4 × MIC or in the presence of **2** (closed squares) at 4 × MIC (mean of two experiments ± standard deviation).

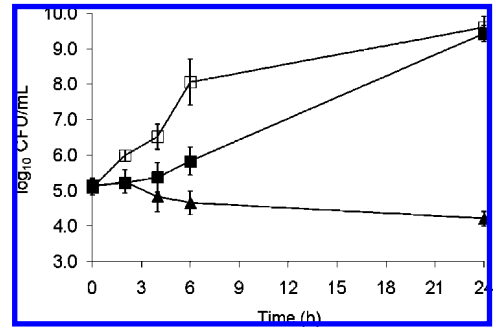

Figure 5. Effect of fructiculine A (**2**) and demethylfructiculine A (**3**) on viable cell number of *E. faecium*. Time-kill curves against *E. faecium* (two VAN-R) in the absence (open squares) or in the presence of **3** (closed triangles) at 4 × MIC or in the presence of **2** (closed squares) at 4 × MIC (mean of two experiments ± standard deviation).

this challenge would be evident. Admittedly, while their total lack of activity on the Gram-negative organisms thus far tested seemingly limits their antimicrobial coverage, it may not prevent their use in association with molecules that display a complementary spectrum (colistin, aztreonam) (*15*).

An icetexane diterpene without a quinone moiety that showed a very modest potency (MIC, 1 mg/mL) against Gram-positive bacteria was isolated from *Salvia lanigera* (*28*). Since the present icetexane compounds **2** and **3** showed a much higher activity (32−64 mg/L), it is inferrable that the quinone moiety might increase the antimicrobial effect of this class of compounds. This is in agreement with the good activity of an abietane diterpene quinone (sanigerone) isolated from *S. lanigera*, which possesses an identical substituted ring C (*29*) and shares the activity shown by various similar diterpene quinones (*30*).

Until now, compounds **2** and **3** had been isolated only from *S. fruticulosa* Benth (*24, 25*). and *S. arizonica* Gray (*26*), and the extractive yield was reported only for the first species: 0.0026% for **2** and 0.01% for **3** (dry weight). As for these two species, the most abundant diterpenoid product isolated from *Salvia corrugata* is demethylfruticuline A. The extractive yields obtained for compounds **2** and **3** from fresh aerial parts were 0.029% and 0.11%, respectively. This result is consistent with the quantitative HPLC determination of **2** and **3**, which showed that the exudate contained 6.5 ± 0.3% of **2** and 33.6 ± 0.8% of **3**, corresponding to theoretical extractive yields of 0.060% and 0.30% from the fresh aerial parts, respectively.

These results and the fact that fresh plant material is easily obtainable in the Mediterranean area point to the potential use

of *Salvia corrugata* as a viable source of these interesting antibacterial substances and, as a consequence, a potential new crop in rural hill and coastal zones.

ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MSSE, Methicillin-susceptible *S. epidermidis*; VAN-S, vancomycin-susceptible; VAN-R, vancomycin-resistant; DMSO dimethyl sulfoxide; MH, Muller Hinton; CFU, colony forming units; MIC, minimal inhibitory concentration; VRSA vancomycin-resistant *Staphylococcus aureus*; CA-MRSA, community acquired methicillin-resistant *Staphylococcus aureus*.

ACKNOWLEDGMENT

We thank Dr. Anna Lanteri for her technical assistance.

LITERATURE CITED

(1) Rodríguez-Hahn, L.; Esquivel, B.; Cárdenas, J.; Ramamoorthy, T. P. In *Advances in Labiate Science*; Harley, R. M., Reynolds, T., Eds.; The Royal Botanic Gardens: Richmond, Surrey, U.K., 1992; pp 335−347.

(2) Duke, S. O. Glandular Trichomes - A Focal Point of Chemical and Structural Interactions. *Int. J. Plant Sci.* **1994**, *155*, 617–620.

(3) Hallahan, D. L. Monoterpenoid Biosynthesis in Glandular Trichomes of Labiatae Plants. In *Advances in Botanical Research*; Hallahan, D. L., Gray, J. C., Callow, J. A., Eds.; Academic Press: San Diego, CA, 2000; Vol. 31, pp 77−111.

(4) Werker, E. 2000. Trichome Diversity and Development. In *Advances in Botanical Research*; Hallahan, D. L., Gray, J. C., Callow, J. A., Eds.; Academic Press: San Diego, CA, 2000; Vol. 31, pp 1−35.

(5) Rodríguez-Hahn, L.; Esquivel, B.; Cárdenas, J. Clerodane Diterpenes in Labiatae. In *Progress in the Chemistry of Organic Natural Products*; Zechmeister, L., Ed.; Springer Verlag: Wien, Österreich, 1994; Vol. 63, pp 107−196.

(6) Kamatou, G. P. P.; Van Vuuren, S. F.; Van Heerden, F. R.; Seaman, T.; Viljoen, A. M. Antibacterial and antimycobacterial activities of South African Salvia species and isolated compounds from *S. chamelaeagnea*. *South Afr. J. Bot.* **2007**, *73*, 552–557.

(7) Weckesser, S. K.; Engel, K.; Simon.Haarhaus, B.; Wittmer, A.; Pelz, K.; Schemp, C. M. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine* **2007**, *14*, 508–516.

(8) Kuzmaa, L.; Rozalskib, M.; Walenckac, E.; Rozalskac, B.; Wysokinska, H. Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclarea* L. Salvipisone as a potential anti-Biofilm agent active against antibiotic resistant Staphylococci. *Phytomedicine* **2007**, *14*, 31–35.

(9) Gonzalez, A. G.; Abad, T.; Jimenez, I. A.; Ravelo, A. G.; Luis, J. G.; Aguiar, Z.; SanAndres, L.; Plasencia, M.; Herrera, J. R.; Moujir, L. A First Study of Antibacterial Activity of Diterpenes Isolated from some *Salvia* Species (Lamiaceae). *Biochem. Syst. Ecol.* **1989**, *17*, 293–296.

(10) Epling, C. A Revision of *Salvia*, subgenus *Calosphace*. In *Repertorium Specierum Novarum Regni Vegetabilis*; Fedde, F., Ed.; Verlag des Repertoriums, Dahlem dei Berlin 1939; University of California Press: Berkley, CA, 1940; Vol. 110, p 51.

(11) Claude, B.; Morin, P. H.; Lafosse, M.; re, P. Evaluation of apparent formation constants of pentacyclic triterpene acids complexes with derivatized β- and γ-cyclodextrins by reversed phase liquid chromatography. *J. Chromatogr. A* **2004**, *1049*, 37–42.

(12) Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover F. C.; Yolken, R. H. *Manual of clinical microbiology*, 7th ed.; ASM Press: Washington, DC, 1999.

(13) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. M100-S16. Wayne, PA: CLSI; **2006**.

(14) Lorian, V. *Antibiotics in Laboratory Medicine*, 5th ed.; Lippincott, Williams and Wilkins: Philadelphia, 2005; pp 28−29.

(15) *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, 5th ed.; Mandell, G., Bennett, J., Dolin, R., Eds.; Churchill Livingstone: Philadelphia, 2005; pp 2147−2152 and 2069−2089.

(16) Schito, G. C. The importance of the development of antibiotic resistance in *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **2006**, *12*, 3–8.

(17) Appelbaum, P. C. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *Int. J. Antimicrob. Agents* **2007**, *30*, 398–408.

(18) Marchese, A.; Schito, G. C. The oxazolidinones as a new family of antimicrobial agent. *Clin. Microbiol. Infect.* **2001**, *7*, 66–74.

(19) Gandelman, G.; Frishman, W. H.; Wiese, C.; Green-Gastwirth, V.; Hong, S.; Aronow, W. S.; Horowitz, H. W. Intravascular device infections: epidemiology, diagnosis, and management. *Cardiol. Rev.* **2007**, *15*, 13–23.

(20) Rice, L. B. Antimicrobial resistance in gram-positive bacteria. *Am. J. Infect. Control* **2006**, *34*, S11−S9 and S64−S73.

(21) Falagas, M. E.; Bliziotis, I. A.; Kasiakou, S. K.; Samonis, G.; Athanassopoulou, P.; Michalopoulos, A. Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. *BMC Infect. Dis.* **2005**, *5*, 24.

(22) Doddrell, D. M.; Khong, P. W.; Lewis, K. G. The stereochemical dependence of ^{13}C chemical shifts in olean-12-enes and urs-12-enes as an aid to structural assignment. *Tetrahedron Lett.* **1974**, *27*, 2381–2384.

(23) Wehrli, F. W.; Nishida, T. The use of Carbon-13 Nuclear magnetic Resonance Spectroscopy. In *Progress in the Chemistry of Organic Natural Products*; Zechmeister, L., Ed; Springer Verlag: Wien, Österreich, 1979; Vol. 36, pp 96−101.

(24) Rodriguez-Hahn, L.; Esquivel, B.; Sánchez, C.; Cárdenas, J.; Estebanes, L.; Soriano-García, M.; Toscano, R.; Ramamoorthy, T. P. New highly oxidized diterpene quinones from *Salvia fruticulosa*. *Tetrahedron Lett.* **1986**, *27*, 5459–5462.

(25) Rodriguez-Hahn, L.; Esquivel, B.; Sánchez, C.; Estebanes, L.; Cárdenas, J.; Soriano-García, M.; Toscano, R.; Ramamoorthy, T. P. Abietane type diterpenoids from *Salvia fruticulosa*. A revision of the structure of fruticulin B. *Phytochemistry* **1989**, *28*, 567–570.

(26) Valant-Vetschera, K. M.; Roitman, J. N.; Wollenweber, E. Chemodiversity of exudates flavonoids in some members of the Lamiaceae. *Biochem. Syst. Ecol.* **2003**, *31*, 1279–1289.

(27) Horiuchi, K.; Shiota, S.; Hatano, T.; Yoshida, T.; Kuroda, T.; Tsuchiya, T. Antimicrobial activity of oleanolic acid from Salvia officinalis and related compounds on vancomycin-resistant enterococci (VRE). *Biol. Pharm. Bull.* **2007**, *30*, 1147–1149.

(28) El-Lakany, A. M.; Abdel-Kader, M. S.; Sabri, N. N.; Stermitz, F. R. Lanigerol: a new antimicrobial icetexane diterpene from *Salvia lanigera*. *Planta Med.* **1995**, *61*, 559–60.

(29) El-Lakany, A. M. Two new diterpene quinones from the roots of *Salvia lanigera* Poir. *Pharmazie* **2003**, *58*, 75–76.

(30) Wellsow, J.; Grayer, R. J.; Veitch, N. C.; Tetsuo, K.; Lelli, R.; Geoffrey, K. C.; Simmonds, M. S. J. Insect-antifeedant and antibacterial activity of diterpenoids from species of *Plectranthus*. *Phytochemistry* **2006**, *67*, 1818–1825.

Received for review May 14, 2008. Revised manuscript received September 16, 2008. Accepted September 17, 2008. The work was performed within the EU INTERREG ALCOTRA project "Developpement des Portentialites du Genre *Salvia*: Nouveaux Produits" n.231.

JF802200X