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Diterpenes from *Salvia broussonetii* Transformed Roots and Their Insecticidal Activity

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The new diterpenes brussonol (**1**) and iguestol (6α,11-dihydroxy-12-methoxy-abieta-8,11,13-triene) (**2**) with an icetexane and a dehydroabietane skeleton, respectively, have been isolated from hairy root cultures of *Salvia broussonetii*. Other previously known diterpenes, 7-oxodehydroabietane, 11-hydroxy-12-methoxyabietatriene, taxodione, inuroyleanol, ferruginol, deoxocarnosol 12-methyl ether, cryptojaponol, pisiferal, sugiol, isomanool, 14-deoxycoleon U, 6α-hydroxydemethylcryptojaponol, demethylsalvicanol, and demethylcryptojaponol, were also obtained from these roots. The insect antifeedant and toxic effects of several of these compounds were investigated against the insect pests *Spodoptera littoralis* and *Leptinotarsa decemlineata*. Additionally, their comparative cytotoxic effects were tested on insect Sf9 and mammalian CHO cells. Demethylsalvicanol (**4**) was a moderate antifeedant to *L. decemlineata*, whereas brussonol (**1**) was inactive. 14-Deoxycoleon U (**15**) was the strongest antifeedant, whereas demethylcryptojaponol (**11**) was toxic to this insect. None of these compounds had antifeedant or negative effects on *S. littoralis* ingestion or weight gains after oral administration. Demethylcryptojaponol (**11**) was cytotoxic to mammalian CHO and insect Sf9 cell lines, followed by the icetexane derivative brussonol (**1**), with moderate cytotoxicity in both cases. The remainder of the test compounds showed a strong selective cytotoxicty to insect Sf9 cells, with demethylsalvicanol (**4**) being the most active.

KEYWORDS: ***Salvia broussonetii*; hairy root cultures; diterpenes; brussonol; iguestol; antifeedants; cytotoxicity**

INTRODUCTION

The genus *Salvia* (Lamiaceae) has been used worldwide in folk medicine since ancient times due to its wide spectrum of activities as an antibacterial (*1*−*4*), antiplasmodial (*5*), antituberculosis, antiphlogistic, cardioactive, antidiabetic (*6*, *7*), anti-inflammatory, analgesic, antipyretic (*8*), antispasmodic (*9*), antitumor (*10*, *11*), antiviral (*12*), hallucinogenic (*13*), trypanocidal (*14*), antifungal (*15*), and antioxidant agent (*16*−*19*). These two last properties are responsible for the fact that sage extracts are used commercially to increase the shelf life of foods. One of the most studied species has been *S. miltiorrhiza*, the rhizomes of which contain tanshinone diterpenes and have been used in China for centuries to treat several diseases. The genus *Salvia* is phytochemically characterized by its diterpene content. The two subgenera *Salvia* and *Sclarea* have abietane diterpenes, whereas *Calosphace* possesses clerodane diterpenes and *Leonia*, abietane and clerodane diterpenes (*20*).

The *Salvia* genus is represented in the Canary Islands by the endemic species *S. canariensis*, *S. broussonetii*, and *S. herbanica*. Previous works have been carried out on the components of *S. canariensis* (*1*, *21*−*25*). The second of these plants, *S. broussonetii* Benth., is an endangered species that grows on the oldest soils of Tenerife island. In earlier studies we isolated the novel triterpenes anagadiol (1β,3β-dihydroxyolean-18-ene) (*26*), nivadiol (3β,11α-dihydroxyolean-18-ene) (*27*), and 3β-hydroxyursa-9(11),12-diene acetate (*28*) from the aerial parts, and the known triterpenes 3-*epi*-maslinic, maslinic, tormentic, and 2α,31,23-trihydroxyolean-12-en-28-oic acids from tissue cultures of this species (*29*). We are interested in the study of the roots, but the scarcity of the plant makes root collection in the field impossible. We have therefore established a hairy root culture of *S. broussonetii* as a renewable source of raw material. The phytochemical study of these roots has now permitted the isolation of two new diterpenes, brussonol (**1**) and iguestol (**2**), as well as other known diterpenes, such as 11-hydroxy-12-methoxyabietatriene (**3**), demethylsalvicanol (**4**), 7-oxodehydroabietane (**5**), ferruginol (**6**), sugiol (**7**), taxodione (**8**), deoxocarnosol 12-methyl ether (**9**), cryptojaponol (**10**), demethylcryptojaponol (**11**), inuroyleanol (**12**), 6α-hydroxydemethyl-

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cryptojaponol (**13**), pisiferal (**14**), 14-deoxycoleon U (**15**), and isomanool (**16**). As a part of our studies on plant defenses, we have tested the antifeedant and insecticidal effects of the diterpenes isolated from the most active fractions against a generalist (*Spodoptera litoralis*) and an oliphagous (*Leptinotarsa decemlipedata*) insect. Additionally, we tested their cytotoxic effects on insect-derived *Spodoptera frugiperda* pupal ovarian cells (Sf9) and mammalian Chinese hamster ovary cell (CHO).

MATERIALS AND METHODS

General Experimental Procedures. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at 500.1 and 125.8 MHz, respectively, with a Bruker AMX-500 spectrometer with pulsed field gradient, using the solvent as an internal standard. Mass spectra were taken in a Micromass Autospect instrument at 70 eV (probe). Dry column chromatographies were made on Merck 0.02−0.063 mm silica gel. When further purification was required, semipreparative HPLC in a Beckman System Gold with a Beckman Ultrasphere Si 1 × 25 cm column was used. Conformations of minimum energy were determined by computational methods employing the Hyperchem 7.1 program of Hypercube.

Plant Material. *Agrobacterium rhizogenes* ATCC-15834 was directly inoculated by a needle to the stem of aseptic plantlets of *S. broussonetti* cultured on agar medium containing 30 g L^{-1} sucrose and half-strength Murashige and Skoog salts (*31*). Roots appeared at the inoculation site after 4 weeks. The induced hairy roots were excised and cultured on hormone-free half-Gamborg B5 solid medium (*31*) supplemented with 30 g L^{-1} sucrose and 0.5 mg mL^{-1} ampicillin to eliminate the bacteria. The axenic hairy roots thus obtained were subcultured in the dark at 25 °C on the same solid medium without antibiotics every 25−30 days.

DNA Extraction and Analysis. Total DNA isolation and PCR analyses were performed by using a REDExtract-N-Amp Seed PCR kit (Sigma). Polymerase Chain Reaction was performed to detect the rolB gene in the genomic DNAs from hairy roots and untransformed roots plants. Plasmid DNA from *A. rhizogenes* strain ATCC-15834 was used as a positive control. The oligonucleotide primers used for amplification of the rolB gene were 5′-ATGGATCCCAAATTGC-TATTCCTTCCA-3′ and 5′-TTAGGCTTCTTTCTTCAGGTTA-3, according to the DNA sequence of the rolB gene described by Furner et al. (*31*). PCR amplification was performed in a DNA thermal cycler (Applied Biosystems 2700) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR reaction mixture was electrophoresed on a 1.2% agarose gel using Tris−acetate−EDTA buffer and visualized by ethidium bromide staining under ultraviolet light at 260 nm.

Culture Method. The hairy roots were cultivated in the dark at 25 °C in 250 mL Erlenmeyer flasks containing 100 mL of half-Gamborg B5 liquid medium (*32*) supplemented with 30 g L^{-1} sucrose and shaken on a rotary shaker at 80 rpm. After 4 weeks, the hairy roots (581 g) were harvested and separated from the culture medium by filtration through filter paper under vacuum.

Extraction and Isolation of Compounds. The hairy roots were freeze-dried (45.3 g), powdered, and extracted with ethanol in a Soxhlet extractor. The ethanolic extract (11.7 g) was evaporated to dryness under reduced pressure, affording a syrupy extract (11.7 g). Then this extract was separated into six fractions by vacuum liquid chromatography (VLC) using a *n*-hexane−EtOAc gradient. All fractions were further rechromatographed on a Si gel column and/or by preparative normal-phase HPLC on an Inertsil Prep-sil (Gasukuro Kogyo) 25 × 2 i.d. column and Ultrasphere Si (Beckman) 25 × 1 i.d. to give, in polarity order, 7-oxodehydroabietane (**5**) (10 mg), 11-hydroxy-12-methoxy-abietatriene (**3**) (55 mg), taxodione (**8**) (6.9 mg), inuroyleanol (**12**) (8 mg), ferruginol (**6**) (5 mg), deoxocarnosol 12-methyl ether (**9**) (4.9 mg), cryptojaponol (**10**) (5.8 mg), pisiferal (**14**) (1.2 mg), iguestol (**2**) (1.5 mg), a mixture of two dimeric substances (8 mg), sugiol (**7**) (2.5 mg), β-sitosterol (39 mg), isomanool (**16**) (3.2 mg), brussonol (**1**) (5 mg), 14-deoxycoleon U (**15**) (16.6 mg), 6α-hydroxydemethylcryptojaponol (**13**) (5.9 mg), demethylsalvicanol (**4**) (16 mg), and demethylcryptojaponol (**11**) (13 mg). (See **Chart 1** for structures.)

Brussonol (1) was obtained as a gum: $[\alpha]_D$ −36.9 (*c* 0.39, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (3H, s, H-19), 0.93 (3H, s, H-18), 1.20 and 1.21 (each 3H, d, *J* = 7 Hz, H-16 and H-17), 1.14 and 1.50 (each 1H, m, H-3), 1.58 (1H, m, H-2), 1.77 (2H, m, H-1α and H-2), 1.79 (1H, d, *J* = 7 Hz, H-5), 1.87 (1H, dd, *J* = 12 and 7 Hz, H-6α), 1.98 (1H, m, H-1β), 2.10 (1H, dt, *J* = 12 and 7 Hz, H-6β), 2.37 (1H, d, *J* = 16 Hz, H-20α), 2.71 (1H, d, *J* = 16 Hz, H-20β), 3.09 (1H, sept, *J* = 7 Hz, H-15), 4.83 (1H, d, *J* = 6.7 Hz, H-7), 6.42 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 316 $[M]^+$ (100), 301 (25), 247 (9), 205 (8), 192 (5), 175 (6), 163 (4); $[M]^+$ at *m*/*z* 316.2030. $C_{20}H_{28}O_3$ requires 316.2038.

Iguestol (2): mp 123−126 °C (petrol−EtOAc), $[\alpha]_D$ +31.8 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (3H, s, H-18), 1.16 (3H, s, H-19), 1.18 and 1.20 (each 3H, d, *J* = 7 Hz, H-16 and H-17), 1.25 (1H, td, *J* = 13 and 3.3 Hz, H-3α), 1.33 (3H, s, H-20), 1.39 (1H, d, *J* = 9 Hz, H-5), 1.45 (1H, dt, *J* = 13 and 4 Hz, H-3β), 1.53 (2H, m, H-1α and H-2), 1.70 (1H, m, H-2), 2.74 (1H, dd, *J* = 15.7 and 7.6 Hz, H-7), 2.99 (1H, dt, *J* = 13 and 4 Hz, H-1β), 3.14 (2H, m, H-7 and H-15), 3.72 (3H, s, −OMe), 4.21 (1H, br signal, $W_{1/2}$ = 18 Hz, H-6), 5.98 (1H, s, OH), 6.45 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 332 $[M]^+$ (100), 317 (10), 299 (59), 267 (28), 257 (11), 229 (48), 193 (10); $[M]^+$ at *m*/*z* 332.2343. $C_{21}H_{32}O_3$ requires 332.2351.

11-Hydroxy-12-methoxyabieta-8,11,13-triene (3): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (3H, s, H-19), 0.96 (3H, s, H-19), 1.19 and 1.21 (each 3H, d, *J* = 7 Hz, H-16 and H-17), 1.24 (1H, m, H-3α), 1.28 (2H, m, H-1 and H-5), 1.33 (3H, s, H-20), 1.46 (1H, dt, *J* = 13 and 3.6 Hz, H-3β), 1.55 (2H, m, H-2 and H-6), 1.74 (1H, ddt, *J* = 13.5 and 3.5 Hz, H-6β), 1.80 (1H, dt, *J* = 12.6 and 3.8 Hz, H-2α), 2.81 (2H, m, 2H-7), 3.17 (2H, m, H-1β and H-15), 3.75 (3H, s, −Ome), 6.00 (1H, s, H0−11), 6.46 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 316 $[M]^+$ (100), 301 (38), 273 (5), 259 (4), 245 (5), 231 (23), 219 (26), 205 (33); $[M]^+$ at *m*/*z* 316.2374. $C_{21}H_{32}O_2$ requires 316.2402.

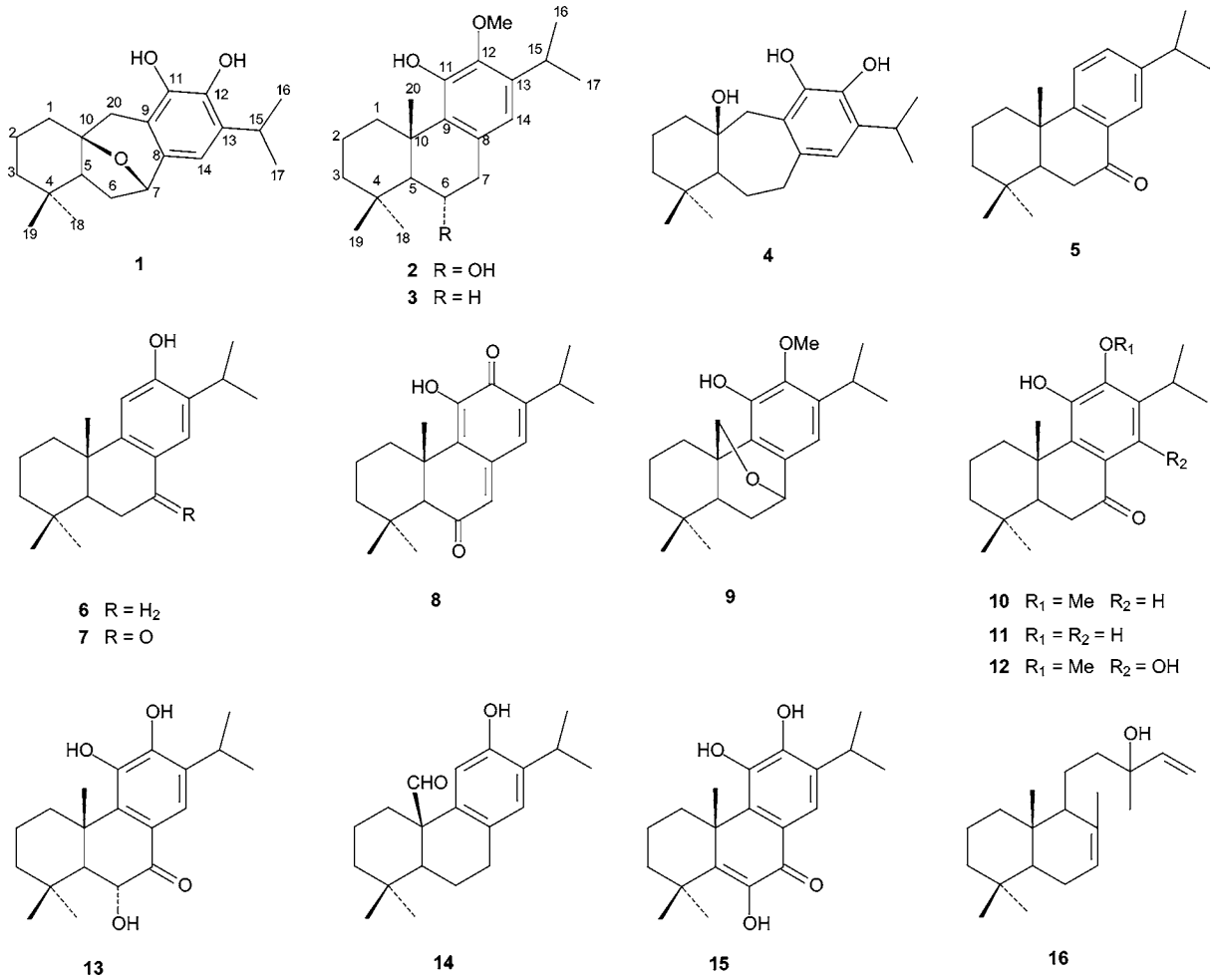
Demethylsalvicanol (4): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, s, H-18), 0.92 (3H, s, H-19), 1.17 and 2.00 (each 1H, m, H-6), 1.33 (1H, d, *J* = 12 and 2.5 Hz, H-5), 1.81 (1H, m, H-2), 2.58 and 3.03 (each 1H, d, *J* = 14.5 Hz, H-20), 2.65 (1H, d, *J* = 15 Hz, H-7α), 2.74 (1H, dd, *J* = 15 and 7 Hz, H-7β), 3.16 (1H, sept, *J* = 7 Hz, H-15), 6.56 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 318 $[M]^+$ (9), 300 (54), 285 (7), 257 (6), 231 (17), 218 (8), 204 (17), 192 (100), 179 (30), 177 (21), 165 (8), 163 (11); $[M]^+$ at *m*/*z* 318.2127. $C_{20}H_{30}O_3$ requires 318.2194.

Deoxocarnosol 12-methyl ether (9): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (3H, s, H-18), 1.15 (3H, s, H-19), 1.21 and 1.22 (each 3H, d, *J* = 7 Hz, H-16 and H-17), 1.45 (1H, m, H-3), 2.06 (2H, m, H-1 and H-6), 2.65 (1H, m, H-1), 3.08 (1H, dd, *J* = 8.5 and 1.7 Hz, H-20), 3.24 (1H, sept, H-15), 3.77 (3H, s, −OMe), 4.32 (1H, d, *J* = 8.5 Hz, H-20), 4.71 (1H, dd, *J* = 3.7 and 1.8 Hz, H-7), 5.84 (1H, s, OH), 6.62 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 330 $[M]^+$ (15), 300 (100), 285 (16), 233 (9), 215 (10), 175 (7); $[M]^+$ at *m*/*z* 330.2170. $C_{21}H_{30}O_3$ requires 330.2194.

Cryptojaponol (10): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (3H, s, H-18), 1.24 and 1.26 (each 3H, d, *J* = 7 Hz, H-16 and H-17), 1.28 (1H, td, *J* = 13.4 and 4 Hz, H-1α), 1.39 (3H, s, H-20), 1.42 (1H, td, *J* = 13 and 4 Hz, H-3α), 1.50 (1H, dt, *J* = 13 and 3 Hz, H-1β), 1.59 and 1.76 (each 1H, m, H-2), 1.87 (1H, dd, *J* = 14.5 and 3 Hz, H-5), 2.55 (1H, dd, *J* = 17 and 14.5 Hz, H-6β), 2.66 (1H, dd, *J* = 17 and 3 Hz, H-6α), 3.19 (1H, sept, *J* = 7 Hz, H-15), 3.24 (1H, dt, *J* = 13.5 and 3 Hz, H-3β), 3.81 (3H, s, −OMe), 6.10 (1H, s, OH), 7.61 (1H, s, H-14); EIMS *m*/*z* (rel intensity) 330 $[M]^+$ (100), 315 (79), 273 (12), 259 (8), 245 (71), 233 (58), 219 (31), 193 (11); $[M]^+$ at *m*/*z* 330.2194. $C_{21}H_{30}O_3$ requires 330.2194.

Inuroyleanol (12): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (3H, s, H-18), 0.96 (3H, s, H-19), 1.36 (3H, s, H-20), 1.27 (1H, td, *J* = 13.8 and 4.2 Hz, H-3α), 1.37 and 1.39 (each 3H, d, *J* = 6.7 Hz, H-16 and H-17), 1.49 (1H, dt, *J* = 13 and 3.8 Hz, H-3β), 1.58 and 1.74 (each 1H, m, H-2), 1.80 (1H, dd, *J* = 10.7 and 6.2 Hz, H-5), 2.63 (2H, m,

Chart 1



H-6), 3.26 (1H, dt, J = 11.0 and 3.8 Hz, H-1β), 3.30 (1H, quint, J = 7 Hz, H-15), 3.78 (3H, s, −OMe), 5.68 (1H, s, HO-11), 13.36 (1H, s, HO-13); EIMS m/z (rel intensity) 346 $[M]^+$ (100), 331 (31), 263 (11), 261 (13), 235 (5), 207 (4); $[M]^+$ at m/z 346.2152. $C_{21}H_{30}O_4$ requires 346.2144.

6α-Hydroxydemethylcryptojaponol (13): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19 (3H, s, H-18), 1.24 (3H, s, H-19), 1.27 and 1.29 (each 3H, d, J = 7 Hz, H-16 and H-17), 1.30 and 1.51 (each 1H, m, H-3), 1.54 (3H, s, H-20), 1.53 (1H, m, H-3), 1.72 (1H, m, H-1), 1.82 (1H, d, J = 13 Hz, H-5), 2.97 (1H, m, H-1), 3.00 (1H, m, H-15), 3.87 (1H, d, J = 2.5 Hz, HO-6), 4.59 (1H, dd, J = 13 and 2.5 Hz), 5.64 and 5.68 (each 1H, br s, HO-11 and HO-12), 7.58 (1H, s, H-14); EIMS m/z (rel intensity) 332 $[M]^+$ (58), 317 (25), 303 (100), 299 (17), 285 (7), 271 (6), 247 (46), 235 (29), 231 (13), 219 (23); $[M]^+$ at m/z 332.1977. $C_{20}H_{28}O_4$ requires 332.1988.

Pisiferal (14): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, s, H-19), 0.98 (3H, s, H-18), 1.16 (1H, m, H-1α), 1.20 and 1.21 (each 3H, d, J = 7 Hz, H-16 and H-17), 1.25 (1H, m, H-3α), 1.45 (1H, dt, J = 13 and 3.5 Hz, H-3β), 1.63 (3H, m, H-5 and 2H-6), 2.07 (2H, m, H-2), 2.85 (1H, dt, J = 12.6 and 3.7 Hz, H-1β), 2.92 (2H, m, H-7), 3.12 (1H, quint, J = 7 Hz, H-15), 6.54 (1H, s, H-11), 6.91 (1H, s, H-14), 9.88 (1H, s, H-20); EIMS m/z (rel intensity) 300 $[M]^+$ (13), 271 (100), 229 (11), 215 (9), 201 (28), 189 (30), 175 (32), 159 (7), 145 (7); $[M]^+$ at m/z 300.2085. $C_{20}H_{28}O_2$ requires 300.2089.

14-Deoxycoleon U (15): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 and 1.30 (each 3H, d, J = 6.8 Hz, H-16 and H-17), 1.41 (1H, m, H-3β), 1.43 and 1.44 (each 3H, s, H-18 and H-19), 1.61 and 1.86 (each 1H, m, H-2), 1.71 and 2.93 (each 1H, m, H-1), 2.01 (1H, td, J = 13 and 5 Hz, H-3α), 3.04 (1H, quint, H-15), 7.09 (1H, s, HO-6), 7.70 (1H, s, H-14); EIMS m/z (rel intensitty) 330 $[M]^+$ (49), 315 (11), 287 (13), 274 (11), 260 (100), 248 (17), 245 (16), 233 (11), 217 (9); $[M]^+$ at m/z 330.1802. $C_{20}H_{26}O_4$ requires 330.1831.

Insect Bioassays. *Insects*. *S. littoralis* and *L. decemlineata* were reared on artificial diet and their respective host plant (*Solanum tuberosum*) and maintained at 22 ± 1 °C, >70% relative humidity, with a photoperiod of 16:8 h (light/dark) in a growth chamber.

Choice Feeding Assays. These experiments were conducted with *S. littoralis* L6 larvae, adult *L. decemlineata*, and apterous aphid adults. Percent feeding inhibition (% FI) and percent settling inhibition (% SI) were calculated as described by Reina (*33*). Compounds with an FR/SI > 50% were tested in a dose−response experiment to calculate their relative potency (EC_{50} values, the effective dose for 50% feeding reduction), which was determined from linear regression analysis (%FR or %SI on log dose).

Oral Cannulation. This experiment was performed with preweighed newly molted *S. littoralis* L6 larvae as previously described (*33*). The possible effect of variations in initial larval weight was analyzed by an analysis of covariance (ANCOVA) performed on biomass gains with initial biomass as covariate. The covariate effect was not significant (p > 0.05), showing that changes in insect biomass were similar among all treatments (*34*, *35*).

Hemolymph Injection. DMSO solutions of the test compounds (10 μg/insect) were injected in 20 adult *L. decemlineata* beetles as described by Reina et al. (*33*). Beetle mortality was recorded for up to 3 days after injection. Percent mortality was analyzed with contingency tables and corrected according to the method of Abbott (*36*).

Cytotoxicity Tests. Sf9 cells, derived from *S. frugiperda* pupal ovarian tissue (ECACC), were maintained in TC-100 insect cell medium supplemented with 10% fetal bovine serum, 1% l-glutamine, and 1% penicillin/streptomycin at 26 °C. Mammalian Chinese hamster ovary cells (CHO) were grown in RPMI 1640 medium supplemented as above at 37 °C under a humified atmosphere of 5% CO_2/95% air.

Cells seeded in 96-well flat-bottom plastic microplates with 100 μL of medium per well (initial densities of 5×10^4 and 10^4 cells per well

for the insect and mammalian cultures, respectively, were exposed for 48 h to serial dilutions of the test compounds. Cell viability was analyzed according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) colorimetric assay method (*37*). A 10 μL aliquot of stock MTT solution (5 mg/mL in PBS) was added to all wells, and the cultures were further incubated for 4 h. The medium was then removed by aspiration, 100 μL of dimethyl sulfoxide was added to dissolve the purple formazan precipitate, and the absorbance at 570 nm (reference wavelength of 630 nm) was measured on a microplate reader (SLT Labinstruments, Groedig, Austria). For each treatment, cell viability was calculated as the percent absorbance of the control (untreated cells). The active compounds were tested in a dose−response experiment to calculate their relative potency (ED_{50} values, the effective dose to give 50% cell viablility), which was determined from linear regression analysis (percent cell viability on log dose).

RESULTS AND DISCUSSION

The structure of the new diterpene brussonol (**1**) was given in accordance with the following considerations. The high-resolution MS showed the base peak and the molecular ion at *m*/*z* 316.2030, indicating a stable structure and a molecular formula of $C_{20}H_{28}O_3$. In the ^{1}H NMR spectrum, signals of the two angular methyls, an isopropyl group, and an aromatic hydrogen could be observed. A pair of doublets at δ 2.37 and 2.71 (J = 16 Hz) was due to H-20, whereas a doublet at δ 4.83 (J = 6.7 Hz) was assigned to H-7, geminal to the oxygen bridge. In the ^{13}C NMR spectrum, signals of four methyls, five methylene groups, four methine groups (C-7 at δ 76.1 and C-14 at δ 112.7), two tetrasubstituted carbons (C-10 at δ 80.0), and five substituted aromatic carbons were seen. Two-dimensional NMR studies (COSY, NOESY, HSQC, and HMBC) permitted the assignment of the ^{1}H and ^{13}C NMR spectra and the establishment of the final structure. Thus, in the HMBC spectrum correlations were observed between H-14 and C-15, C-7, C-9, and C-12, between H-7 and C-10, C-14, and C-9, between H-20 and C-7, C-5, C-10, C-9, C-8, and C-11, and between H-6 and C-4, C-5, C-7, and C-8.

A plausible biogenetic relation is given in **Scheme 1** for the formation of compounds **1**, **4**, **21**, and **22**, all found in *Salvia* species. Biosynthetically, brussonol (**1**) can derive from demethylsalvicanol (**4**). The latter should be formed by enzymatic abstraction of a hydrogen from a C-20 methyl or by solvolysis of a C-20 hydroxyl group in compound **17** or **18**, respectively, to afford the carbenium ion **19**, which is neutralized by a hydroxyl group, giving **4**. We have assigned the β-stereochemistry to the 7,10-ether bridge of **1**, considering that it is formed by attack of the C-10 hydroxyl over the C-7 of a product (**20**) formed by oxidation of **4**. This stereochemistry is in accordance with the form of resonance and coupling constant for H-7 (d, J = 6.7 Hz). Thus, the lower energy conformation obtained by computational methods showed angles of H-7 with H-6α of 89.8° and with H-6β of 31.2°. Demethylsalvicanol (**4**) (*38*) has also been isolated from this species, and its ^{1}H and ^{13}C NMR spectra have been unambiguously assigned using 2D NMR methods.

Brussonol (**1**) represents a biosynthetic step between demethylsalvicanol (**4**) and 5,6-dihydro-6α-hydroxysalviasperanol (**21**), which should also be named 6α-hydroxybrussonol. This compound by biodehydration can form salviasperanol (**22**) (**Scheme 1**). The diterpenes **21** and **22** had been isolated from the roots of *S. aspera*, a Mexican species (*39*).

The second new diterpene isolated from the roots was **2**, which we have named iguestol. Its HRMS indicated a molecular formula of $C_{21}H_{32}O_3$. The ^{13}C NMR (**Table 3**) spectrum

Scheme 1

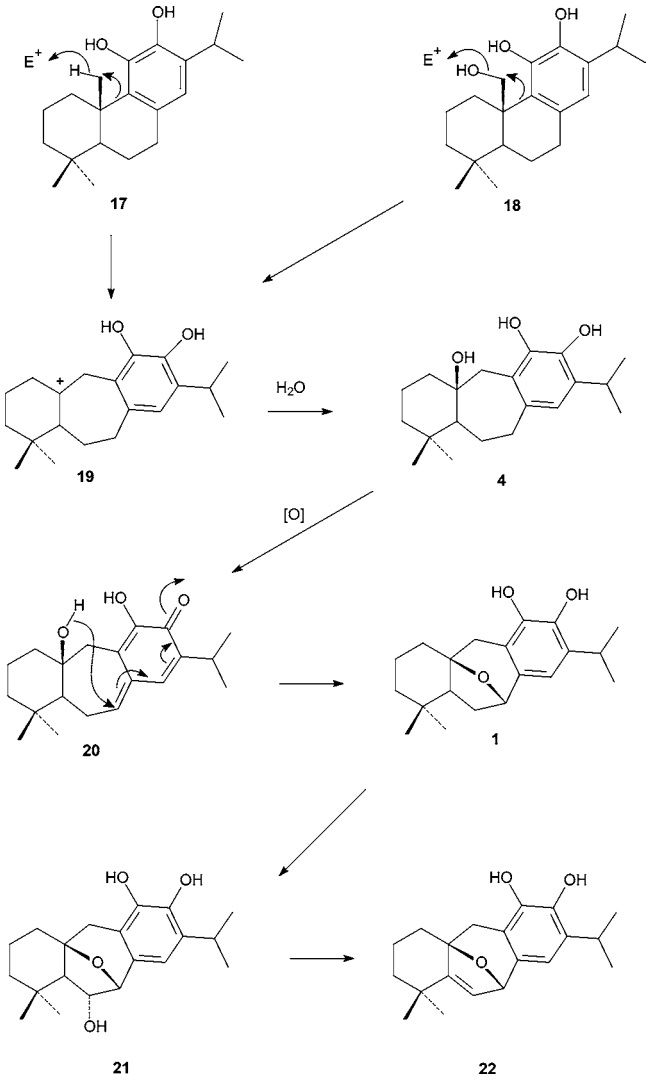


Table 1. Effective Antifeedant Doses (EC_{50} and 95% Confidence Limits) and Mortality (72 h) Data Corrected According to the Method of Abbot (*36*) of the Test Compounds on Adult *L. decemlineata* and Consumption (ΔI) and Biomass Gain (ΔB) of Orally Injected *S. littoralis* L6 Larvae

	L. decemlineata		*S. littoralis* (% of control)	
compd	EC_{50} (μg/cm^2)	% mortality (72 h)	ΔI	ΔB
1	>100	7	na[a]	na
3	10.2 (4.8, 22.2)	14	91	102
11	>100	43[b]	103	105
12	>100	0	119	106
15	1.8 (0.5,6.6)	29	107	99

[a] Insufficient compound available. [b] Significantly different from the control, P < 0.05, contingency table analysis.

indicated the existence in the molecule of five methyls, a methoxy group, four methylenes, three methines, two tetrasubstituted carbons, and six aromatic carbons (one unsubstituted and five substituted). The ^{1}H NMR spectrum showed three angular methyls, an isopropyl group, a methoxy group, and a proton geminal to a secondary alcohol. Other signals observed in this spectrum were two singlets at δ 5.98 and 6.45, which were assigned to a phenolic hydroxyl group, associated with the oxygen of the methoxy group, and to an aromatic hydrogen, respectively.

Table 2. Cytotoxic Effects on Sf9 and CHO Cells of the Test Compounds

compd	LD_{50}[a] (μg/mL) Sf9	CHO
1	19.97 (14.09, 28.30)	50.64 (34.94, 73.40)
3	0.15 (0.07, 0.36)	>100
11	0.64 (0.38, 1.08)	36.03 (20.74, 62.60)
13	0.98 (0.80, 1.21)	>100
15	0.45 (0.29, 0.69)	>100

[a] Effective dose to give a 50% cell viability.

Table 3. ^{13}C NMR Data ($CDCl_3$) of Compounds **1**–**4** and **12**–**16**

C	1	2	3	4	12	13	14	15	16
1	30.7	37.7	36.5	41.6	36.5	37.1	32.6	30.3	39.2
2	16.1	19.1	19.2	18.7	19.0	18.9	18.3	17.8	18.8
3	32.2	42.6	41.6	42.3	41.2	41.9	41.3	36.3	42.3
4	31.8	34.0	33.7	34.4	33.4	34.0	33.8	36.4	33.1
5	51.0	58.2	52.9	58.1	49.7	55.0	51.7	143.2	50.1
6	39.6	68.5	19.4	24.3	35.9	73.0	19.6	140.7	23.7
7	76.1	42.2	31.9	36.0	206.1	200.1	30.0	179.8	122.2
8	134.2	131.8	133.4	136.4	112.5	122.3	130.5	120.1	135.3
9	116.4	131.1	133.1	120.3	135.9	132.2	133.6	138.1	55.2
10	80.0	41.5	39.3	71.3	40.3	41.0	52.1	40.6	37.0
11	141.5	146.0	146.9	142.6	138.6	141.0	113.8	142.9	21.2
12	139.4	143.0	142.4	140.4	152.2	146.8	151.5	145.3	44.9
13	131.9	138.0	137.6	132.4	126.1	132.2	134.2	132.6	73.6
14	112.7	117.3	117.5	117.5	158.3	118.1	127.2	116.4	145.1
15	27.1	26.3	26.4	27.2	26.0	27.3	26.9	27.0	111.7
16	22.5	23.5	23.7	21.5	20.3	22.3	22.4	22.3	27.4
17	22.8	23.6	23.8	22.3	20.4	22.3	22.5	22.5	22.2
18	30.6	35.6	33.8	32.2	33.1	22.4	31.5	27.3	33.1
19	26.6	22.9	22.1	22.8	21.6	22.8	20.5	27.9	21.7
20	38.7	21.2	19.9	41.6	17.9	19.9	200.7	27.9	13.5

All of these data and the HBMC spectrum (see below) indicated a dehydroabietane skeleton for **2**, with a secondary alcohol, a phenolic group, and a methoxy group. The resonance form of the geminal hydrogen to the hydroxyl group (δ 4.21, br signal) and the low resonance value of the corresponding carbon (δ 68.5) permitted the assignment of this alcohol group at C-2 or C-6. The latter position was chosen on the basis of 2D NMR data, which also permitted assignment to C-11 and C-12 of the phenolic and methoxy groups, respectively. Thus, in the HMBC spectrum correlations were observed between H-14 and C-15, C-7, C-9, and C-12, between HO-11 and C-9, C-12, and C-11), betwen 12-OMe and C-12, between H-15 and C-16, C-17, C-14, C-13, and C-12, between H-7 and C-5, C-6, C-14, and C-8], between H-1 and C-2 and C-10, between H-5 and C-20, C-1, and C-3, between H-20 and C-1, C-10, C-5, and C-9, between H-16 and H-17 and C-15, between H-18 and C-19, C-4, C-3, and C-5, and between H-19 and C-18, C-4, C-3, and C-5. The stereochemistry of the hydroxyl group was determined as α-equatorial on the basis of the broad signal of its geminal proton ($W_{1/2} = 18$ Hz) and the coupling observed with H-5 (δ 1.39, d, $J = 9$ Hz). Additionally, cross-peaks of H-6 with H-19 and H-20 were observed in its NOESY spectrum. Therefore, the structure of iguestol was determined as 6α,11-dihydroxy-12-methoxy-abieta-8,11,13-triene (**2**).

Other diterpenes isolated from the hairy root cultures from this plant were 7-oxodehydroabietane (**5**) (*40*), 11-hydroxy-12-methoxyabietatriene (**3**) (*41*), taxodione (**8**) (*42*−*44*), inuroyleanol (**12**) (*45*), ferruginol (**6**) (*46*), deoxocarnosol 12-methyl ether (**9**) (*25*), cryptojaponol (**10**) (*47*), pisiferal (**14**) (*48*), sugiol (**7**) (*44*, *49*), isomanool (**16**) (*50*), 14-deoxycoleon U (**15**) (*44*, *47*), 6α-hydroxydemethylcryptojaponol (**13**) (*25*), demethylsalvicanol (**4**) (*38*), and demethylcryptojaponol (**11**) (*44*, *47*). The ^{13}C NMR spectra of those compounds that have been unambiguously assigned are indicated in **Table 3**.

The isolation of pentacyclic triterpenes (*27*−*29*) from the aerial part and of diterpenes from the hairy roots of *S. broussonetii* is interesting. Another species of this genus with a similar pattern in the distribution of these terpenes is *S. argentea* (*51*, *52*). Therefore, the differential location of diterpenes and triterpenes within the plant may be related to plant maturation or defense allocation. This fact can be used as a chemotaxonomical marker, also considering that both species show a similar morphology.

None of the test compounds was antifeedant against *S. littoralis* (data not shown). The icetexane diterpene demethylsalvicanol (**4**) was a moderate antifeedant to *L. decemlineata*, whereas brussonol (**1**), with a similar skeleton, was inactive, suggesting a correlation between this activity and the absence of the C-7/C-19 oxygen bridge in **4** (**Table 1**). The dehydroabietane 14-deoxycoleon U (**15**) was the strongest antifeedant diterpene (**Table 1**), suggesting a possible role for the unsaturation of the B-ring (**15** versus **13**). Additionally, demethylcryptojaponol (**11**) was moderately toxic to this insect. None of these compounds had a negative effect on *S. littoralis* ingestion or weight gains after oral administration (**Table 1**). Abietane diterpene acids had strong dose-dependent antifeedant effects on the lepidopteran *Lymantria dispar* (*53*).

Table 2 shows the cytotoxic effects of these compounds on insect and mammalian cells. Demethylcryptojaponol (**11**) was cytotoxic to both cell lines, followed by brussonol (**1**) with moderate cytotoxicity in both cases. The remainder of the test compounds showed a strong selective cytotoxicity to insect Sf9 cells, with demethylsalvicanol (**4**) being the most active. This selective cytotoxic effect of **4**, **13**, and **15** may be due to membrane-related factors, and the lack of in vivo toxicity to *S. littoralis* suggests metabolic detoxification. The icetexane komaroviquinoine, structurally similar to **1** but with a C-7 hemiketal, has been reported as a trypanocidal agent (*14*). Some abietane diterpenes isolated from *S. milthiorrhiza* have cytotoxic effects against tumor cell lines (*54*, *55*). However, this is the first report on insect cytotoxic effects for this class of compounds.

This selective insect cytotoxic effect suggests a defensive role of these compounds in *S. broussonetii* roots against generalized herbivores in the presence of potential inhibitors of the insect's metabolic detoxification system, such as antioxidants (*56*). Abietane diterpenes with ortho-dihydroxy groups exhibited strong antioxidant activity (*18*) and therefore could act as natural synergists of co-occurring insecticidal compounds.

LITERATURE CITED

(1) González, A. G.; Andrés, L. S.; Herrera, J. R.; Luis, J. G.; Ravelo, A. G. Abietane diterpenes with antibiotic activity from the flowers of *Salvia canariensis*. Reaction of galdosol with diazomethane. *Can. J. Chem.* **1989**, *67*, 208−212.

(2) Moujir, L.; Gutiérrez-Navarro, A. M.; San Andrés, L.; Luis, J. G. Structure−antimicrobial activity relationships of abietane diterpenes from *Salvia* species. *Phytochemistry* **1993**, *34*, 1493.

(3) El-Lakany, A. M.; Abdel-Kader, A.; Sabri, N. H.; Stermitz, F. R. Lanigerol: a new antimicrobial icetexane diterpene from *Salvia lanigera*. *Planta Med.* **1995**, *61*, 559−560.

(4) Ulubelen, A.; Öksüz S.; Topcu, G.; Gören, A. C.; Voelter, W. Antibacterial diterpenes from the roots of *Salvia blepharochlaena*. *J. Nat. Prod.* **2001**, *64*, 549−551.

(5) Sairafianpour, M.; Christensen, J.; Staerk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J. W. Leishmanicidal, antiplasmodial and cytotoxic activity of novel diterpenoid 1,2-quinones from *Perovskia abrotanoides*: new source of thanshinones. *J. Nat. Prod*. **2001**, *64*, 1398−1403.
(6) Ulubelen, A.; Topen, G.; Johansson, C. B. Norditerpenoids and diterpenoids from *Salvia multicaulis* with antituberculosis activity. *J. Nat. Prod*. **1997**, *60*, 1275−1280.
(7) Ulubelen, A. Cardioactive and antibacterial terpenoids from *Salvia* species. *Phytochemistry* **2003**, *64*, 395−399.
(8) Hernández-Pérez M.; Rabanal, R. M.; De la Torre, M. C.; Rodríguez, B. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives. *Planta Med*. **1995**, *61*, 505−509.
(9) Romussi, G.; Ciarallo, G.; Bisio, A.; Fontana, N.; De Simone, F.; De Tommasi, N.; Mascolo, N.; Pinto, L. A new diterpenoid with antispasmodic activity from *Salvia cinnabarina*. *Planta Med*. **2001**, *67*, 153−155.
(10) Zhang, J. S.; Ding, J.; Tang, Q. M.; Li, M.; Zhao, M.; Lu, L. J.; Chen, L. J.; Yuan, S. T. Synthesis and antitumour activity of novel diterpenequinone salvicine and the analogs. *Bioorg. Med. Lett.* **1999**, *9*, 2731−2736.
(11) Lin, H. C.; Chang, W. L. Diterpenes from *Salvia miltiorrhiza*. *Phytochemistry* **2000**, *53*, 951−953.
(12) Tada, M.; Okuno, K.; Chiba, K.; Ohnishi, E.; Yoshi, T. Antiviral diterpenes from *Salvia officinalis*. *Phytochemistry* **1994**, *35*, 539−541.
(13) Valdés, L. J.; Chang, H. M.; Visger, D. C.; Koreeda, M. Salvinorin C, a new neoclerodane diterpene from a bioactive fraction of the hallucinogenic Mexican mint *Salvia divinorum*. *Org. Lett*. **2001**, *3*, 3935−3937.
(14) Uchiyama, N.; Kiuchi, F.; Ito, M.; Honda, G.; Takeda, Y.; Khodzhimatov, O. K.; Asurmetov, O. A. New icetexane and 20-norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi*. *J. Nat. Prod*. **2003**, *66*, 128−131.
(15) Pitarokili, D.; Colaudis, M.; Petsikos-Panayotarou, N.; Tzakou, O. Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. *J. Agric. Food Chem*. **2002**, *50*, 6688−6691.
(16) Weng, X. C.; Gordon, M. H. Antioxidant activity of quinone extracted from Tanshen (*Salvia miltiorrhiza* Bunge). *J. Agric. Food Chem*. **1992**, *40*, 1931−1936.
(17) Cuvelier, M. E.; Berset, C.; Richard, H. Antioxidant constituents in sage (*Salvia officinalis*). *J. Agric. Food Chem*. **1994**, *42*, 665−669.
(18) Miura, K.; Kikuzaki, H.; Nakatani, N. Antioxidant activity of chemical components of sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) measured by the oil stability index method. *J. Agric. Food. Chem*. **2002**, *50*, 1845−1851.
(19) Matsingou, T. C.; Petrakis, N.; Kapsokefalou, M.; Salifoglou, A.; Antioxidant activity of organic extracts from aqueous infusions of sage. *J. Agric. Food Chem*. **2003**, *51*, 6696−6701.
(20) Rodriguez-Hahn, L.; Esquivel, B.; Cárdenas, J.; Ramamoorthy, T. The distribution of diterpenoids in *Salvia*. In *Advances in Labiatae Science*; Harley, R. M., Reynolds, T., Eds.; Royal Botanic Gardens: Kew, U.K., 1992; pp 335−347.
(21) González, A. G.; Fraga, B. M.; Luis, J. G.; Ravelo, A. G. Structure of galdosol, a new diterpene from *Salvia canariensis*. *Experientia* **1973**, *29*, 1471.
(22) González, A. G.; Fraga, B. M.; Luis, J. G.; Ravelo, A. G. Componentes de la *Salvia canariensis*. *Anal. Quim*. **1975**, *71*, 701−705.
(23) González, A. G.; Rodríguez, C. M.; Luis, J. G. Diterpenes from the flowers of *Salvia canariensis*. *Phytochemistry* **1987**, *26*, 1471−1474.
(24) Fraga, B. M.; González, A. G.; Herrera, J. R.; Luis, J. G.; Perales, A.; Ravelo, A. G. A revised structure for the diterpene rosmanol. *Phytochemistry* **1985**, *24*, 1853−1854
(25) Fraga, B. M.; González, A. G.; Herrera, J. R.; Luis, J. G.; Ravelo, A. G. Diterpenes from the roots of *Salvia canariensis*. *Phytochemistry* **1986**, *25*, 269−271.
(26) González, A. G.; Bretón, J. L.; Fraga, B. M. Constituents of Labiatae. Anagadiol, a new triterpene from *Salvia broussonetii* Benth. *Chem. Commun*. **1971**, 567−568.
(27) González, A. G.; Bretón, J. L.; Fraga, B. M. Estructura del nivadiol, un nuevo triterpeno de la *Salvia broussonetii* Benth. *Anal. Quim*. **1972**, *68*, 709−722.
(28) González, A. G.; Fraga, B. M.; Ravelo, A. G. Triterpenos de la *Salvia broussonetii* Benth. *Anal. Quim*. **1972**, *68*, 1433−1436.
(29) Fraga, B. M.; Diaz, C. E.; Valdes, F.; Carmona, A. Triterpenos en cultivo de tejidos de *Salvia broussonetii*. *Anal. Quim*. **1991**, *87*, 929−930.
(30) Murashige, T.; Skoog, F. Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. **1962**, *15*, 473−479.
(31) Furner, I. J.; Huffman, G. A.; Amasino, R. M.; Garfinkel, D. J.; Gordon, M. P.; Nester, E. W. An *Agrobacterium* transformation in the evolution of the genus *Nicotiana*. *Nature* **1986**, *319*, 422−427.
(32) Gamborg, O. L.; Miller, R. A.; Ojima, K. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res*. **1968**, *50*, 151−158.
(33) Reina, M.; González-Coloma, A.; Gutiérrez, C.; Cabrera, R.; Rodriguez, M. L.; Fajardo, V.; Villarroel, L. Defensive chemistry of *Senecio miser* Hook. *J. Nat. Prod.* **2001**, *64*, 6−11.
(34) Horton, R. D.; Redak, R. A. Further comments on analysis of covariance in insect dietary studies. *Entomol. Exp. Appl*. **1993**, *69*, 263−275.
(35) Raubenheimer, D.; Simpson, S. J. Analysis of covariance: an alternative to nutritional indices. *Entomol. Exp. Appl*. **1992**, *62*, 221−231.
(36) Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.
(37) Mossman, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(38) Kelecom, A.; Medeiros, W. L. B. A novel rearranged abietane diterpene from *Coleus barbatus*. *Bull. Soc. Chim. Belg*. **1989**, *98*, 413.
(39) Esquivel, B.; Flores, M.; Hernández-Ortega, S.; Toscano, R.; Ramamoorthy, T. P. Abietane and icetexane diterpenoids from the roots of *Salvia aspera*. *Phytochemistry* **1995**, *39*, 139−143.
(40) Norin, T.; Winell, B. Diterpenoids of cones from two *Cedrus* species. *Phytochemistry* **1971**, *10*, 2818−2821.
(41) Ulubelen, A.; Topcu, G.; Tan, N. Rearranged abietane diterpenes from *Salvia candissima*. *Phytochemistry* **1992**, *31*, 3637−3638.
(42) Kupchan, S. M.; Karin, A.; Marcks, C. Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from *Taxodium distichum*. *J. Org. Chem*. **1969**, *34*, 3912−3918.
(43) Haslinger, E.; Michl, G. Synthese von (+)-taxodion. *Liebigs Ann. Chem*. **1989**, 677−686.
(44) Rodriguez, B. ^{1}H and ^{13}C NMR spectral assignments of some natural abietane diterpenoids. *Magn. Reson. Chem*. **2003**, *41*, 741−746.
(45) Bhat, S. V.; Kalyanaraman, P. S.; Kohl, H.; De Souza, N. J.; Ferhlhaber, H. W. Inuroyleanol and 7-ketoroyleanone, two novel diterpenoids of *Inula royleana* DC. *Tetrahedron* **1975**, *31*, 1001−1004.
(46) Brandt, C. W.; Neubauer, L. G. Miro resin. I. Ferruginol. *J. Chem. Soc*. **1939**, 1031−1037.
(47) Hueso-Rodriguez, J. A.; Jimeno, M. L.; Rodríguez, B.; Savona, S.; Bruno, M. Abietane diterpenoids from the root of *Salvia phlomoides*. *Phytochemistry* **1983**, *22*, 2005−2009.
(48) Yatagai, M.; Takahashi, T. New diterpenes from *Chamaecyparis pisifera*. *Phytochemistry* **1980**, *19*, 1149−1151.

(49) Chang, H. M.; Cheng, K. P.; Choang, T. F.; Chow, H. F.; Chui, K. Y.; Hon, P. M.; Tan, F. W. L.; Yang, Y.; Zhong, Z. P.; Lee, C. M.; Sham, H. L.; Chan, C. F.; Cui, Y. X.; Wong, H. N. C. Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* Bunge (Danshen). *J. Org. Chem*. **1990**, *55*, 3527−3543.

(50) Torii, S.; Uneyama, K.; Kawahara, I.; Kuyama, M. A synthesis of diastereomers of (±)-labda-7,14-dien-13-ol. *Chem. Lett*. **1978**, 455−456.

(51) Michavilla, A.; De la Torre, M. C.; Rodríguez, B. 20-Nor-abietane diterpenes and rearranged abietane diterpenoids from the root of *Salvia argentea*. *Phytochemistry* **1986**, *25*, 1935−1937.

(52) Bruno, M.; Savona, G.; Hueso-Rodríguez, J. A.; Pascual, C.; Rodríguez, B. Ursane and oleane triterpenoids from *Salvia argentea*. *Phytochemistry* **1987**, *26*, 497−501.

(53) Powell, J. S.; Raffa, K. F. Effects of selected *Larix laricinia* terpenoids on *Lymantria dispar* (Lepidoptera: Lymantridae) development and behavior. *Environ. Entomol*. **1999**, *28*, 148−154.

(54) Ryu, S. Y.; Lee, C. O.; Choi, S. U. In vitro cytotoxicity of tanshinones from *Salvia miltiorrhiza*. *Planta Med*. **1997**, *63*, 339−342.

(55) Chen, X.; Ding, J.; Ye, Y.-M.; Zhang, J. S. Bioactive abietane and *sec*-abietane diterpenoids from *Salvia prionitis*. *J. Nat. Prod*. **2002**, *65*, 1016−1020.

(56) Berenbaum, M. Brementown. Revisited: interactions among allelochemicals in plants. In *Recent Advances in Phytochemistry*; Cooper-Driver, G. A., Swain, T., Conn, E. E., Eds.; Plenum Press: New York, 1984; Vol. 19, p 139.

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