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## METHYL β-ORCINOLCARBOXYLATE AND ATRANOL FROM THE LICHEN *STEREOCAULON VESUVIANUM*

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Extracts of *Stereocaulon vesuvianum* Pers. showed strong activity against *Bacillus subtilis* and *Escherichia coli* and a distinct activity against *Penicillium digitatum* and *Saccharomyces cerevisiae*. By preparative hplc, methyl β-orcinolcarboxylate (strongly antifungal) and atranol (strongly antibacterial) were isolated, and it was shown that both compounds are present as natural products and are not artifacts formed during work-up. Methyl β-orcinolcarboxylate was recently suggested to be present in *S. vesuvianum* by the revision (1) of an isomeric structure previously reported (2) in this species. Sizable amounts of the common cortical depside atranorin (inactive) were also isolated.

### EXPERIMENTAL

PLANT MATERIAL.—*S. vesuvianum* was collected on the middle slopes (ca. 1300 m) of Mt. Etna. It grows abundantly only on the ancient (2-3 centuries old) lava flows. A voucher specimen is deposited at the University of Catania.

EXTRACTION AND ISOLATION OF METHYL β-ORCINOLCARBOXYLATE, ATRANOL, AND ATRANORIN.—The air-dried and ground lichen thalli (100 g) were extracted by stirring at room temperature with 500 ml of *n*-hexane. The extract was concentrated in vacuo (49 mg), and it was fractionated by hplc on a Hypersil column 25 cm×3.9 mm i.d. (eluent *n*-heptane-iPrOH, 95:5). Methyl β-orcinolcarboxylate and atranol, in order of elution, were identified by spiking their peaks with authentic materials and by mass spectral comparisons. Thus, the presence of these compounds derives from the plant material, although the yield from this hexane extraction is low due to their limited solubility. These compounds are not artifacts formed during work-up (3) or due to improper extraction solvent (i.e., $CHCl_3$ stabilized with EtOH) that can cause alcoholysis of atranorin, as recently cautioned (1). Atranorin may serve as a storage compound from which the plant slowly produces the other two bioactive compounds to protect itself from attack by pathogenic microorganisms.

A similar extraction of 100 g of the lichen in $CH_2Cl_2$, stabilized with amylene, yielded a residue (807 mg) that, after washing with *n*-hexane, was identified as atranorin (0.56% yield). Methyl β-orcinolcarboxylate and atranol were also obtained in the hexane washing. All compounds were identified by direct comparison (hplc, ms, $^1H$ nmr) with authentic samples.

To isolate atranol, 500 g of *S. vesuvianum* were extracted with $H_2O$ by heating at 120° at 1.2 kg/cm$^2$ in a sterilizing autoclave for 30 min. The obtained aqueous suspension was filtered and partitioned with $CHCl_3$. The $CHCl_3$ concentrate (0.907 g) was stirred with $CH_2Cl_2$-*n*-hexane (2:1). Preparative hplc of the dissolved material (0.481 g) on a Magnum Partisil column 25 cm× 9 mm id (eluent *n*-hexane-*t*-butylmethylether, 75:25) gave, besides methyl β-orcinolcarboxylate (105 mg), atranol (60 mg) which was identified by comparison of the mp and ir spectrum with those reported in the literature (4, 5).

*Atranol.*—(2,6-Dihydroxy-4-methylbenzaldehyde) uv λ max nm (ε) 280 (12600), 349 (2600); $^1$H nmr 10.29 (1H, s, CHO), 8.84 (2H, broad OH), 6.22 (2H, s, H3 and H5), 2.27 (3H, s, $CH_3$); eims (probe) 70 eV *m/z* (rel. int.) 152 ($M^+$, 91), 151 (100), 136 (3), 123 (2), 106 (6), 95 (4); all data previously unreported in the literature.

Antimicrobial activity of atranol, obtained using the agar plate disc diffusion method, (μg applied), mm zone of inhibition: against *B. subtilis* (ATCC 6633) (40), 17; streptomycin sulfate as control (0.6), 17; against *E. coli* (strain B, ATCC 11303) (40), 17; streptomycin sulfate as control (6), 25; against *S. cerevisiae* (baker yeast) (40) 18; filipin as control (24), 24. Details of the procedure have been previously reported (6).

The antimicrobial activity of methyl β-orcinolcarboxylate isolated from another lichen has been reported (7).

Full details of the isolation and identification of the compounds are available on request to the senior author.

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# FLAVONOIDS FROM *SALVIA NICOLSONIANA*

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As part of a systematic chemical investigation of Mexican Labiatae (1-4), we previously reported several triterpenoids from *Salvia nicolsoniana* Ramamoorthy (2). Continuing our studies of this genus, a new collection of this species was analyzed and resulted in the isolation and characterization of six known flavonoids. The chemistry of *S. nicolsoniana* does not differ from the chemical profile outlined for this genus, since it contains pentacyclic triterpenes and flavonoids which are widely distributed metabolites among the members of Labiatae (4).

## EXPERIMENTAL

PLANT MATERIAL.—*S. nicolsoniana* was collected in Sierra Madre del Sur, Guerrero, México, in February 1985. Reference specimens are deposited in the National Herbarium, Instituto de Biología de la Universidad Nacional Autónoma de México, voucher No. 6191-M.

EXTRACTION AND ISOLATION PROCEDURES.—Exhaustive chromatography of the $Me_2CO$ extract of the dried aerial parts (7 kg) yielded four terpenoids, namely betulinic, oleanolic, ursolic, and 3α,24 dihydroxyolean-12-en-28-oic acids, as well as β-sitosterol, which were identical in all respects (mp, tlc, ir, ms, $^1$H nmr) with authentic samples (2,5,6). In addition, six flavonoids were also isolated from this species in the following order from Si gel column chromatography: apigenin 4,7′-dimethyl ether (12 mg), isosakuranetin (37.8 mg), acacetin (859.3 mg), genkwanin (17 mg), cirsimaritin (15.2 mg), and luteolin 3′,4′-dimethyl ether (7 mg).