Materials and Methods

W. somnifera was cultivated in the Botanical Garden of the University "La Sapienza" of Rome from seeds of plants from Sicily collected in the Palermo neighbourhood. Sardinian plants were collected in the Nuoro neighbourhood. Sicilian plants were identified by Prof. B. Anzalone, Univ. "La Sapienza", and the Sardinian ones by Prof. L. Mossa, Univ. of Cagliari. Voucher specimens (WsNM 216) are deposited in the Herbarium of the Dip. di Biologia Vegetale, Rome.

Leaves (100 g), collected during the flowering period, were extracted with MeOH (1 l) at room temperature for 5 days. After evaporation of the solvent, the residue (ca. 1 g) was separated by CC on silica gel 60 (800 g) in toluene/EtOAc (3 ml/min) with increasing percentages (95 : 5, 90 : 10, 80 : 20, each fraction 100 ml) obtaining pure withanolides, namely **1** (120 mg, maximum after 100 ml), **2** (40 mg, maximum after 160 ml), **3** (20 mg, maximum after 200 ml), **4** (10 mg, maximum after 220 ml), and **5** (50 mg, maximum after 240 ml), in increasing polarity order, detection of eluates by TLC (SiO_2 in toluene/EtOAc as previously reported; 2 N H_2SO_4 at 110 °C as spray reagent). Analogous separation was performed for Sardinian plants, giving **1** (175 mg), **2** (50 mg), **3** (30 mg), **5** (25 mg), and **6** (70 mg), and for the roots of the examined plants, without isolation of withanolides as evidenced by TLC analyses. Withanolides were identified by comparison of their ^{1}H- and ^{13}C-NMR spectra (Bruker AM500; $CDCl_3$; TMS as internal reference) with literature data (1, 3–5). Only ^{13}C-NMR data of 5 are reported here, since they were not previously published: δ = 203.9 (C-1), 166.2 (C-26), 150.2 (C-24), 138.6 (C-3), 129.6 (C-2), 121.5 (C-25), 88.2 (C-17), 82.9 (C-14), 81.3 (C-22), 80.0 (C-20), 79.1 (C-5), 69.4 (C-6), 54.4 (C-13), 49.7 (C-10), 37.7 (C-12), 34.1 (C-9), 34.1 (C-16), 33.9 (C-8), 32.7 (C-23), 30.6 (C-4), 29.9 (C-15), 27.6 (C-7), 22.6 (C-11), 21.0 (C-18), 20.5 (C-28), 20.1 (C-21), 15.5 (C-19), 12.3 (C-27). The HPLC system consisted of an HP 1090L chromatograph equipped with a Lichrospher RP-18 (5 mm I.D., length 25 cm) column; U.V. detector at 214 and 335 nm. The gradient elution was performed at 25 °C with mobile phase A (H_2O): B (CH_3CN). The gradient programme started at sample injection and was linear from 25 % to 100 % B for the solvent concentration and from 0.38 to 0.80 ml/min for the flow in a total period of 51 min.

Axillary explants of Sicilian plants were grown for 60 days according to Heble (6). Shoots cultured on different media were weighed every 10 days. Roots in micropropagated plants were induced by supplement of IBA (4 mg/l) or IAA (1 mg/l). Stems of different-aged seedlings were inoculated with an exponential culture of *A. rhizogenes* LBA 9402, agropine type (7). Hairy roots grown in liquid and solid MS medium showed characteristic plagiotropism and lateral branching, and transformation was demonstrated by hybridization with pRi 1855 EcoRI SmaI fragment 15 (8).

References

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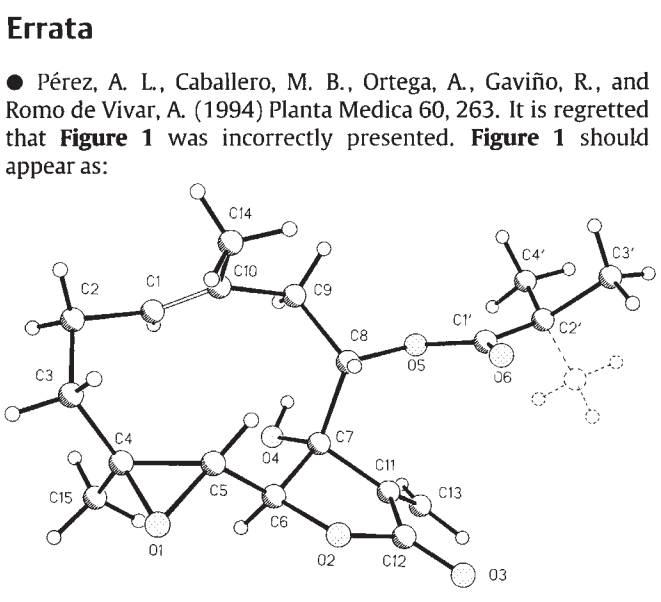
Errata

● Pérez, A. L., Caballero, M. B., Ortega, A., Gaviño, R., and Romo de Vivar, A. (1994) Planta Medica 60, 263. It is regretted that **Figure 1** was incorrectly presented. **Figure 1** should appear as:



● The diterpene lanigerol published as a new compound in the paper entitled "Lanigerol: a new anti microbial icetexane diterpene from *Salvia lanigera*", *Planta Medica 1995*, **61**, 559, was previously isolated from *Chamaecyparis pisifera* family Cupressaceae under the name pisiferanol (10S, 12-dihydroxy-9(10,20)-abeo-abieta-8, 11, 13-triene), *Phytochemistry* 1985, **24** (7), 1545–1551. This is its second isolation from nature and first from family Labiatae.

● Cimanga, K., De Bruyne, T., Lasure, A., Van Poel, B., Pieters, L., Claeys, M., Vanden Berghe, D., Kambu, K., Tona, L., and Vlietinck, A. J. (1966) Planta Medica 62, 22–27. Some NMR data for compounds 1 and 4 were incorrectly given. The correct data are as follows:

Quindoline (**1**): ^{1}H-NMR (CD_3OD, 200 MHz): δ = 8.33 (1H, m, H-6 or H-4), 8.09 (1H, s, H-11), 8.05 (1H, m, H-4 or H-6), 7.89 (1H, m, H-1), 7.60–7.35 (4H, m), 7.17 (1H, m), (H-2, H-3, H-7, H-8, H-9). ^{13}C-NMR (CD_3OD, 50 MHz): δ = 131.22, 128.60, 128.60, 127.78, 126.26 (C-1, C-2, C-3, C-4, C-8), 122.87 (C-6), 120.83 (C-7), 115.22 (C-11), 112.42 (C-9) (Quaternary signals were not observed due to their low intensity).

Cryptolepine (**4**): ^{1}H-NMR (CD_3OD, 200 MHz): δ = 8.51 (1H, s, H-11), 8.2–8.0 (3H, m, H-1, H-4, H-6), 7.83 (1H, m, H-3), 7.59 (1H, m, H-9), 7.45 (2H, m, H-2, H-8), 6.98 (1H, m, H-7), 4.59 (3H, s, N-CH_3). ^{13}C-NMR (CD_3OD, 50 MHz): see Table **4**.