Phytochemistry, Vol. 28, No. 3, pp. 849–850, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

A SESQUITERPENE ALCOHOL FROM *PALLENIS SPINOSA*

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(*Received* 24 *June* 1988)

Key Word Index—*Pallenis spinosa*; Compositae; germacrane alcohols; 2D NMR.

Abstract—The aerial parts of *Pallenis spinosa* gave a new germacrane alcohol, whose structure and conformation were established by spectral data, including 2D NMR.

INTRODUCTION

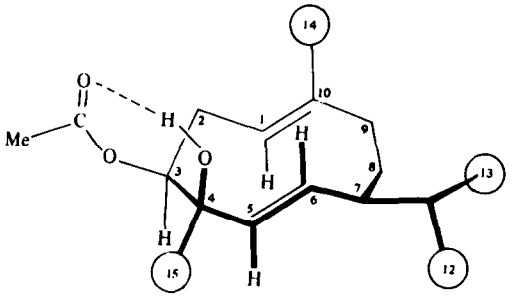
Three species from the small Mediterranean genus *Asteriscus* (tribe Inuleae) have been studied so far [1–3]. The results obtained showed that oxygenated humulane derivatives are characteristic for the genus. Since oxygenated humulanes are rare in plants from the Compositae family, this pattern is taxonomically relevant.

We have now studied *Pallenis spinosa* (L.) Cass., a plant of uncertain taxonomical position also known as *Asteriscus spinosus* G. et G. [4], to assess whether the phytochemical pattern could justify the inclusion of this plant in a genus different from *Asteriscus*. No other plants from the genus *Pallenis* have been studied so far.

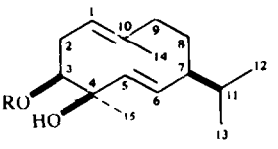
RESULTS AND DISCUSSION

The major constituent from a chloroform extract of the aerial parts was the crystalline germacrane alcohol **1a**. The constitution of **1a** was deduced from its ^1H and ^{13}C NMR spectra, using a combination of mono- and bidimensional techniques. The ^1H–^1H coupling pattern of the molecule was analysed by the COSY spectrum in its phase-sensitive double-quantum filtered version; this technique is nowadays recognized as the most effective to obtain well-resolved cross-peaks allowing a correct evaluation of the coupling constants between the protons involved. Furthermore, the double-quantum filtering allows a good suppression of strong singlets (e.g. methyl groups, solvent) on the diagonal, leading to a better recognition of the underlying signals [5, 6].

The complete and unambiguous assignment of the ^{13}C resonances was achieved by a ^1H–^{13}C 2D chemical shift correlation [7]. Simple chemical shift considerations and inspection of literature data did not allow us to distinguish between some sets of signals (δ 135.19 and 127.31 for C-5 and C-6; 23.74 and 30.05 for C-2 and C-8; 18.97, 20.56 and 26.82 for C-12, C-13 and C-15), whereas a straightforward assignment was possible on simple inspection of the splitting pattern of the ^1H traces (f_1 domain) obtained at the corresponding ^{13}C frequencies (f_2 domain). The relative stereochemistry and the conformation in solution were deduced as follows: the ^1H NMR spectrum of **1a** at room temperature consisted of sharp lines, and some



a



1a R = Ac
1b R = H

coupling constants between vicinal annular protons were significantly high (> 10 Hz; $J_{1,2\beta} = J_{2\beta,3} = 12.3$ Hz; $J_{6,7} = 10.7$ Hz). Therefore **1a** is monorotameric in solution, since a dynamic conformational process involving the medium-size ring would have resulted in either the appearance of broad peaks or averaged values of J's [8]. The configuration at the endocyclic double bonds followed from the observation of the relevant ^1H($J_{5,6} = 15.6$ Hz) and ^{13}C- (δC-14 = 16.90) NMR data [9]. The acetyl group at C-3 was placed equatorial on account of the splitting pattern of H-3 (*dd*, $J_{2\beta,3} = 12.3$ Hz; $J_{2\alpha,3} = 4.2$ Hz). The stereochemistry at the quaternary centre C-4 and at C-7 was deduced from the results of NOE experiments (NOE H-3, H-15 = 12%; NOE H-3, H-5 = 22%) and inspection of $J_{5,6}$ (10.7 Hz). The presence of a relevant (19%) NOE between H-6 and H-14

showed that the conformation of **1a** in solution is of the [$_1$D^{14}, $_5$D^6] type [10], with the double bonds parallel (A).

Saponification (NaOMe) of the acetyl group gave the diol **1b**. In this compound H-3 was also coupled to the proton on the secondary hydroxyl ($J = 7.8$ Hz), suggesting the presence of a strong intramolecular hydrogen bonding between the two vicinal hydroxyls. In **1a** the hydrogen bonding most probably involves the carbonyl oxygen as acceptor (see A), as suggested by IR ($\nu_{C=O} = 1710\ cm^{-1}$) spectroscopy.

The presence of a germacrane alcohol and the absence of oxygenated humulanes clearly distinguishes the chemistry of the plant from that of the genus *Asteriscus*, supporting its inclusion in a different genus (*Pallenis*).

EXPERIMENTAL

NMR: 300 MHz and 75.1 MHz for ^{1}H and ^{13}C respectively. A 0.07 M soln. of **1a** in $CDCl_3$ was used. NOE's were measured 0.6 sec. (mixing time) after a selective 180° pulse on the proton under examination. Double-quantum filtered phase-sensitive COSY was run according to refs [5, 6]. 256 traces were collected for each of the two matrixes (states hypercomplex method, 16 transients per trace; digital resolution: 3.5 Hz/point in both Fourrier transformations). ^{1}H–^{13}C chemical shift correlation was run according to ref. [7]. Two different spectra were run for low- and high-field resonances. 128 transients were accumulated for the 256 traces (digital resolution: 9.8 Hz/point). The digital resolution in the second dimension was 1.4 Hz/point.

Silica gel 60 (70–230 mesh, Merck) was used for CC. *P. spinosa* was collected in Orosei (NU, Sardinia) in June 1987, and was identified by V.P.

Isolation of **1a**. 663 g of non-woody aerial parts (leaves and flowers) were extracted with $CHCl_3$ at room temp., affording 13 g of crude extract, which was dissolved in EtOH (200 ml) and then treated with a 4% $Pb(OAc)_2$ aq. soln (200 ml). After removal of the ppt. the filtrate was diluted with H_2O and extracted with $CHCl_3$, to give 1.38 g of a dark gum. The latter was chromatographed on a column of silica gel (20 g) eluted with $CHCl_3$. The least polar fractions were rechromatographed on 20 g silica gel (eluent hexane–EtOAc 19:1) to give **1a** (320 mg) as a greenish solid (yield: 0.05%). Crystallization from hexane at low temp. (−5°), afforded shining needles.

[3S*, 4R*, 7S*, 1(10)E, 5E] 3-*Acetoxygermacra*-1(10),5-*dien*-4-*ol* (**1a**). Colourless needles, mp 113°, $[\alpha]_D^{25}$ −145 (CH_2Cl_2, *c* 0.80); IR ν_{max}^{KBr} cm^{-1}: 3500, 1710, 1675, 1375, 1270, 1200, 1030, 975; EIMS 70 eV, *m/z* (rel. int.): 280 $[M]^+$ ($C_{17}H_{28}O_3$) (11); 220 $[M-60]^+$ (14); 81 (95); 71 (22); 43 (100); ^{1}H NMR (300 MHz, $CDCl_3$, TMS as reference): δ 5.35 (*dd*, $J_{5,6} = 15.6$ Hz, $J_{6,7} = 10.7$ Hz, H-6), 5.16 (*d*, $J_{5,6} = 15.6$ Hz, H-5), 5.02 (*br d*, $J_{1,2\beta} = 12.3$, H-1), 4.76 (*dd*, $J_{2\beta,3} = 12.3$ Hz, $J_{2\alpha,3} = 4.2$ Hz, H-3), 2.70 (*d t*, $J_{2\alpha,2\beta} = 14.3$ Hz, $J_{1,2\beta} = J_{2\beta,3} = 12.3$ Hz, H-2), 2.10 (*s*, OAc), 1.54 (*br s*, H-14), 1.16 (*s*, H-15), 0.82, 0.78 (*d*, $J = 6.1$, H-12 and H-13); ^{13}C NMR (75.1 MHz, $CDCl_3$, TMS as reference): δ 170.32 (*s*, OAc), 135.19 (*d*, C-5), 133.93 (*s*, C-10); 127.31 (*d*, C-6), 125.14 (*d*, C-1), 76.67 (*d*, C-3), 74.64 (*s*, C-4); 52.81 (*d*, C-7), 41.03 (*t*, C-9), 33.03 (*d*, C-11), 30.05 (*t*, C-2), 26.82 (*q*, C-15), 23.74 (*t*, C-8), 21.16 (*s*, OAc), 20.56 and 18.97 (*q*, C-12 and C-13), 16.90 (*q*, C-14).

[3S*, 4R*, 7S*, 1(10)E, 5E] *Germacra*-1(10),5-*dien*-3,4-*diol* (**1b**). Crude **1a** (145 mg) was dissolved in MeOH (1 ml), and 1 M NaOMe (1 ml) was added. After stirring 15 min under N_2, the soln. was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was washed with brine and evapd, giving a semi-solid residue, which was crystallized from hexane, to give **1b** (42 mg) as leaflets, mp 128°, $[\alpha]_D^{25}$ −201 (CH_2Cl_2, *c* 1.3); IR ν_{max}^{KBr} cm^{-1}: 3520, 3300, 1670, 1455, 1440, 1370, 985, 945, 845, 830; ^{1}H NMR (300 MHz, C_6D_6, TMS as reference): δ 5.32 (*dd*, $J_{5,6} = 15.5$ Hz, $J_{6,7} = 9.7$ Hz, H-6), 4.89 (*br d*, $J_{1,2} = 11.4$ Hz, H-1), 4.87 (*d*, $J_{5,6} = 15.5$ Hz, H-5), 3.13 (*ddd*, $J_{2\beta,3} = 11.4$ Hz, $J_{2\alpha,3} = 4.2$, $J_{3,OH} = 7.8$ Hz, H-3), 2.54 (*dt*, $J_{1,2\beta} = J_{2\beta,3} = 11.4$, $J_{2\alpha,2\beta} = 13.2$, H-2$\beta$), 1.19 (*br s*, H-14); 0.88, 0.84 (*d*, $J = 6.1$ Hz, H-12 and H-13); ^{13}C NMR (75.1 MHz, $CDCl_3$, TMS as reference): δ 136.55 (*d*, C-5), 133.01 (*s*, C-10), 125.97 (*d*, C-6), 125.51 (*d*, C-1), δ 75.53 (*s*, C-5), 74.93 (*d*, C-3), 53.09 (*d*, C-7), 41.08 (*t*, C-9), 33.86 (*t*, C-2), 33.11 (*d*, C-11), 26.85 (*q*, C-15), 23.71 (*t*, C-8), 20.57 and 18.95 (*q*, C-12 and C-13), 17.00 (*q*, C-14).

Acknowledgements—This work was supported by the Ministero della Pubblica Istruzione (fondi 40% and 60%). We are grateful to Prof. C. Bicchi and C. Frattini and to Dr A. D'Amato for the mass spectra, and to Prof. G. M. Nano for his interest in this work.

REFERENCES

1. San Feliciano, A. S., Barrero, A. F., Medarde, M., Miguel de Corral, J. M., Aramburu Aizpiri, A. and Sánchez-Ferrando, F. (1984) *Tetrahedron* **40**, 873.
2. El-Dahmy, S., Jakupovic, J., Bohlmann, F. and Sarg, T. M. (1985) *Tetrahedron* **41**, 309.
3. Jakupovic, J., Lehmann, L., Bohlmann, F. and Arroyo Hogdson, A. (1987) *Phytochemistry* **26**, 2854.
4. Pignatti, S. (1982) in *Flora d'Italia*, Vol. III, p. 52. Edagricole, Bologna.
5. Piantini, V., Sørensen, O. W. and Ernst, R. R. (1982) *J. Am. Chem. Soc.* **104**, 6800.
6. Rance, M., Sørensen, O. W., Bodenhauser, G., Wagner, G. and Ernst, R. R. (1983) *Biochem. Biophys. Res. Commun.* **117**, 479.
7. Joyce, A., Bolton, W. and Bolton, P. H. (1984) *J. Magn. Res.* **59**, 343.
8. Appendino, G., Valle, M. G. and Gariboldi, P. (1986) *J. Chem. Soc. Perkin I*, 1363.
9. Lange, G. L. and Lee, M. (1986) *Magn. Reson. Chem.* **24**, 656.
10. Samek, Z. and Harmatha, J. (1978) *Collect. Czech. Chem. Commun.* **43**, 2779.