Phytochemistry, 1975, Vol. 14, pp. 2247–2248. Pergamon Press. Printed in England.

STEREOCHEMISTRY OF GRANILIN ISOLATED FROM *CARPESIUM ABROTANOIDES*

MASAO MARUYAMA and FUMIKO SHIBATA

Department of Chemistry, Miyagi University of Education, Sendai, Japan

(*Received* 25 *January* 1975)

Key Word Index—*Carpesium abrotanoides*; Compositae; granilin.

Abstract—Granilin was obtained from *Carpesium abrotanoides* as an antibacterial component, and its relative stereochemistry was established.

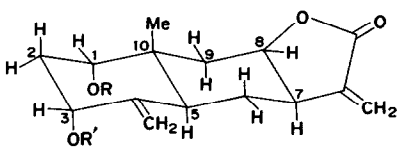
We have initiated a systematic search for components of plants active against *Cochliobolus miyabeanus* (Ito et Kurib.) Drechsl. and *Xanthomonus oryzae* (Uyeda et Ishiyama) Dowson in order to obtain new antifungal and antibacterial substances from plant sources. This paper reports the isolation from *Carpesium abrotanoides* Linn. (Compositae) of granilin [1] and elucidation of its stereochemistry. Other chemical investigations on this plant have been reported [2,3].

Extraction with MeOH of the whole plant afforded a green gum, which showed *in vitro* activity against *C. miyabeanus* and *X. oryzae*. Solvent partition followed by silica gel chromatography of the crude extract guided by biological assay afforded several active fractions, one of which on recrystallization gave an active crystalline substance which was identified as granilin (**1**) by direct comparison with authentic material.

Nikonova *et al.* [1] have reported the structure of granilin (**1**) as 1,3-dihydroxyeudesma-4(14), 11(13)-dien-7,8-olide with a *cis*-fused lactone ring. In our study, analysis of the NMR spectrum and decoupling experiments combined with the NMR shift reagent technique, revealed the stereochemistry of granilin (**1**) at C-1, C-3, C-5 and C-10 shown in structure **1**.

In the NMR spectrum of **1** in DMSO-d_6 the C-1 and C-3 protons give rise to broad signals at 3·32 ($W_{1/2}$ = 5 Hz) and 4·23 ppm ($W_{1/2}$ = 4Hz) respectively, which are explicable only in terms of the axial orientation of the two hydroxyl groups [4]. This conclusion is in accord with the frequency of the carbonyl absorption in the IR spectrum of the monoacetate (**2**) and the diacetate (**3**) obtained on acetylation of **1**. The acetyl carbonyl of **3** absorbed at 1732 cm^{-1} while that of **2** absorbed at 1760 cm^{-1}. This unusually high frequency can be ascribed to intramolecular hydrogen bonding between the axial hydroxyl and the acetoxyl groups [5].

The stereochemistry of the ring junction at C-5 and C-10 was assumed by Nikonova [1] to be *trans* by analogy to other eudesmanolides. This was proved unambiguously by comparing the NMR spectra of **2** with and without $Eu(dpm)_3$ as a shift reagent. While the signals of the protons at C-1, C-2α, C-5 and C-9α shifted downfield by 1·97, 1·18, 1·06 and 1·35 ppm, respectively, on addition of 1/4 mol of $Eu(dpm)_3$ to the $CDCl_3$ soln of **2**, no other signal was shifted by more than 0·7 ppm. This suggests that the hydroxyl group to which the shift reagent coordinates is at C-1, and that C-2α, C-5 and C-9α hydrogens are located close to the C-1 hydroxyl group. These relationships can only be derived from the C-5 and C-10 *trans*-fused eudesmanolide structure shown in structure **1**.



(**1**) R = R′ = H
(**2**) R = H, R′ = Ac
(**3**) R = R′ = Ac

EXPERIMENTAL

Mps are uncorrected. NMR were measured with TMS as internal standard at 100 MHz, MS were by the direct inlet method. Biological assays were carried out by the paper disk method on incubated agar surfaces.

Granilin (**1**). The conc. MeOH extract (1 kg) from 21 kg of undried plant was partitioned between $CHCl_3$ (2·4 l.) and H_2O (2·4 l.). The H_2O layer was washed with $CHCl_3$ (1 litre) and the combined $CHCl_3$ extract was washed with H_2O. Evaporation of the $CHCl_3$ gave a dark green tar (247 g) which contained most of the antifungal and antibacterial activity present in the MeOH extract. The green tar was partitioned between 5% aq MeOH (300 ml) and petrol (200 ml) and the aq MeOH fraction was washed with petrol (5 × 200 ml portions).

The aq MeOH soln was conc. to a dark brown tar (90 g) which showed activity. Si gel (1·6 kg) column chromatography (elution with $CHCl_3$ and $CHCl_3$–MeOH) of the tar (34 g) gave 15 fractions (fractions A–O). Fraction K (2·32 g) eluted with $CHCl_3$–MeOH, 99:1, was semicrystalline. Recrystallization with Me_2CO afforded granilin (**1**) (1·15 g) as colourless prisms, mp 188–191°, $[\alpha]_D^{21}$ +157° (MeOH, *c*, 1·02), NMR (DMSO-d_6): δ 0·68 (3H, *s* C-10 Me), 1·24 (1H, *q*, *J* 13 Hz, C-6β), 1·70 (1H, *br d*, *J* 16 Hz, C-9β), 1·7 (1H, *m*, C-6α), 1·86 (2H, *t*, *J* 3 Hz, C-2), 2·39 (1H, *dd*, *J* 16 and 5 Hz, C-9α), 2·66 (1H, *br d*, *J* 13 Hz, C-5), 3·15 (1H, *m*, C-7), 3·32 (1H, *br*, C-1), 4·23 (1H, *br*, C-3), 4·59 (1H, *br*, C-14), 4·65 (1H, *br t*, *J* 5 Hz, C-8), 4·96 (1H, *br*, C-14), 5·76 (1H, *s*, C-13), 6·00 (1H, *s*, C-13), which was characterized by mmp, TLC and IR spectra comparisons with authentic material.

Acetylation of **1**. Treatment of **1** (100 mg) in boiling Ac_2O (5 ml) with NaOAc (100 mg) for 5 min followed by PLC (Si gel G, $CHCl_3$–MeOH, 19:1) and recrystallization from EtOH afforded the monoacetate (**2**) as colourless needles (37 mg), mp 154–156; $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1760; NMR ($CDCl_3$): δ 0·84 (3H, *s*, C-10 Me), 1·41 (1H, *dt*, *J* 14 and 12 Hz, C-6β), 1·81 (1H, *ddd*, *J* 14, 7 and 3 Hz, C-6α), 1·91 (1H, *dd*, *J* 16 and 2 Hz), 2·08 (3H, *s*, OAc), 2·1 (2H, *m*, C-2), 2·50 (1H, *dd*, *J*, 16 and 5 Hz, C-9α), 2·69 (1H, *br d*, C-5), 3·03 (1H, *m*, C-7), 3·43 (1H, *m*, C-1), 4·62 (1H, *dt*, *J*, 2 and 5·5 Hz, C-8), 4·84 (1H, *br*, C-14), 5·24 (1H, *br*, C-14), 5·51 (1H, *t*, *J* 3Hz, C-3), 5·60 (1H, *s*, C-13), 6·16 (1H, *s*, C-13); NMR ($CDCl_3$) (with 1/4 mol $Eu(dpm)_3$); δ 1·30 (3H, *s*, C-10 Me), 1·18 (1H, *q*, *J* 13 Hz, C-6β), 2·20 (3H, *s*, OAc), 2·2 (1H, *m*, C-6α), 2·44 (1H, *br*, *d*, *J* 15 Hz, C-9β), 2·80 (1H, *dt*, *J* 16 and 3 Hz, C-2β), 3·28 (1H, *m*, C-2α), 3·28 (1H, *m*, C-7), 3·75 (1H, *br d*, *J* 13 Hz, C-5), 3·85 (1H, *dd*, *J* 15 and 4 Hz, C-9α), 4·75 (1H, *dd*, *J* 6 and 4 Hz, C-8), 5·18 (1H, *br*, C-14), 5·40 (1H, *m*, C-1), 5·60 (1H, *br*, C-14), 5·70 (1H, *s*, C-13), 6·20 (1H, *m*, C-3), 6·24 (1H, *s*, C-13); MS: *m/e* 264, 246 (Found: C, 66·55; H, 7·33. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24%), and noncrystalline diacetate (**3**) (66 mg), $\nu_{max}^{CHCl_3}$ cm^{-1}: 1764, 1732. NMR ($CDCl_3$): δ 2·05 (3H, *s*, OAc), 2·13 (3H, *s*, OAc), 4·65 (1H, *m*, C-1), 5·40 (1H, *m*, C-3).

Acknowledgements—We are indebted to Mitsui Toatsu Chemicals, Inc. for biological assays and financial support. We also wish to thank Mr. J. Ishiyama and Mrs. R. Tanaka of Tohoku University for NMR measurements and Microanalysis Laboratory of Tohoku University for elemental analysis, and to Dr. L. P. Nikonova for an authentic sample of granilin.

REFERENCES

1. Nikonova, L. P. and Nikonov, G. K. (1972) *Khim. Prir. Soedin.* **8,** 289.
2. Naito, S. (1955) *J. Pharm. Soc.* **75,** 93, 325.
3. Minato, H., Nosaka, S. and Horibe, I. (1964) *J. Chem. Soc.* 5503.
4. Herz, W., Subramanian, P. S. and Geissman, T. A. (1968) *J. Org. Chem.* **33,** 3743.
5. West, R., Korst, J. J. and Johnson, W. S. (1960) *J. Org. Chem.* **25,** 1976.