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AURANTIAMIDES: A NEW CLASS OF MODIFIED DIPEPTIDES FROM *PIPER AURANTIACUM*

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Key Word Index—*Piper aurantiacum*; Piperaceae; seeds; modified dipeptides; aurantiamide, dia-aurantiamide and their acetates.

Abstract—Two new amides, aurantiamide and aurantiamide acetate, were isolated from *Piper aurantiacum*. Their structures were determined as *N*-(*N'*-benzoyl-*S*-phenylalaninyl)-*S*-phenylalaninol and its acetate, respectively, from chemical and spectroscopic studies. The structures and stereochemistry were confirmed by synthesis. The corresponding diastereoisomers were also synthesized and their spectroscopic properties compared with those of the natural compounds.

INTRODUCTION

Plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic system of medicine for their medicinal properties [1, 2]. Previous investigations of some of these plants have led to the isolation of a number of physiologically active compounds. A variety of compounds including amide derivatives, flavones, chalcones, kawa-pyrones, lignans, sterols, terpenes, amino acids, vitamins and carbohydrates have been previously isolated from different *Piper* species [1, 3, 4]. In continuation of our work on *Piper* species [5–7], we undertook the investigation of the seeds of *Piper aurantiacum* collected from Assam. The alcoholic extract of the fruits of *P. aurantiacum* are reported [4] to possess hypotensive activity and cause strong stimulation of the uterus and intestines, increasing both tone and movement.

Chemical investigation of the seeds of *P. aurantiacum* has led to the isolation of two new amides, aurantiamide and aurantiamide acetate, in addition to some known non-nitrogenous compounds [8]. The structures of the new amides have been determined from their chemical and spectral data and confirmed by synthesis [9]. Other workers have recently reported [10, 11] the isolation of the compounds piperine, piperettine, sylvatine, β-sitosterol, cholesterol and cholestanol from the same plant.

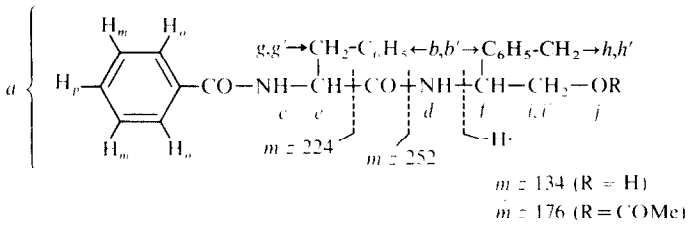
RESULTS AND DISCUSSION

Aurantiamide, $C_{25}H_{26}N_2O_3$ (M^+ 402.1890, calc. 402.1838), a neutral compound, did not exhibit any characteristic UV absorption. It could not be catalytically hydrogenated, which showed the absence of any olefinic unsaturation in the compound. Its IR absorption spectrum (KBr) showed the presence of –NH– and/or –OH groups (broad band at 3280 cm^{-1}), amide carbonyl (1650 and 1625 cm^{-1}) and monosubstituted benzene (1597, 1573, 755 and 695 cm^{-1}) moieties. The 80 MHz ^1H NMR ($CDCl_3$) spectrum confirmed the presence of the above groups, showing signals for two –CONH– groups (δ 6.70, 1 H, *d*, J = 8 Hz and δ 5.93, 1 H, *d*, J = 8 Hz), an OH (δ 3.20–3.45, partially overlapped with the signals of a methylene group), 15 aromatic protons incorporated in a benzoyl (δ 7.28–7.41, 3 H, *m* and δ 7.60, 2 H, *d* of *d*; J_o = 7 Hz, J_m = 2 Hz) and two benzyl (δ 7.02, 5 H, *br s* and δ 7.16, 5 H, *br s*) groups. All the signals in the ^1H NMR spectrum could be unequivocally assigned only after the structure of aurantiamide had been established from its high resolution mass spectrum.

The 20 MHz ^{13}C NMR spectrum (d_6-DMSO) of aurantiamide showed the presence of two carbonyls (δ 166.2 and δ 170.9) in addition to 3 methylenes (δ 62.3, δ 36.5 and δ 37.3) and two methines (δ 52.5 and δ 54.8). It was apparent from their chemical shifts that the three low-field aliphatic carbons were situated next to hetero-atoms.

The second compound, aurantiamide acetate, $C_{27}H_{28}N_2O_4$ (M^+ 444), was originally designated PA-A. The IR, ^1H NMR and mass spectra of PA-A were very similar to those of aurantiamide. In the IR spectrum additional bands appeared at 1725 and 1260 cm^{-1}, whereas in the ^1H NMR spectrum, there was an additional 3-proton singlet at δ 1.98, instead of the OH proton. Its ^{13}C NMR spectrum (d_6-DMSO) was also similar (two N–CH< at δ 54.9 and δ 49.2, an –O–CH_2– at δ 64.7 and two benzylic methylenes at δ 37.3 and δ 36.7), but showed the presence of an extra carbonyl (δ 169.8, δ 171.2 and δ 166.2) and a Me group (δ 20.6). Mild hydrolysis of PA-A afforded aurantiamide, and aurantiamide on acetylation with acetic anhydride/pyridine yielded PA-A. Hence PA-A was the acetate of aurantiamide.

The structures of aurantiamide and PA-A, without any stereochemical implications, were established as **1** and **2** respectively from a consideration of the above data and a detailed analysis of their mass spectral fragmentation patterns (including high-resolution studies). The mass spectra showed that they possessed modified dipeptide structures, derived from *N*-benzoylphenylalanine and phenylalaninol. The diagnostic fragmentations are shown on the structural formulae **1** and **2**.



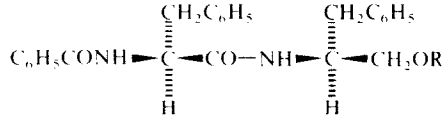
1 R = H, aurantiamide
2 R = COMe, aurantiamide acetate

The ^{1}H NMR spectra (see Experimental) of these compounds were fully in accordance with their assigned structures. In both aurantiamide and aurantiamide acetate some of the methylene protons were found to be magnetically equivalent and the others non-equivalent. The difference in the behaviour of the different methylene groups is certainly due to some conformational rigidity imposed on the molecule through intramolecular hydrogen bonding. The assignments have been confirmed by the following double-resonance experiments with aurantiamide acetate: protons *c* and *d* collapsed to singlets on irradiation of *e* and *f*, respectively; proton *e* changed to a doublet of doublets ($J_1 = 8$ Hz, $J_2 = 6$ Hz) on irradiation of *c* and to a doublet ($J = 7.5$ Hz) on irradiation of *g*, *g'*; irradiation of *e* changed the two doublet of doublets due to *g*, *g'* to an AB quartet ($J = 13.5$ Hz); the doublet due to protons *h* collapsed to a singlet on irradiation of *f*.

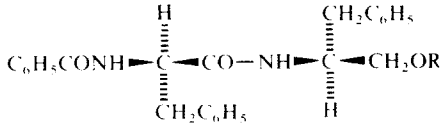
Four stereoisomeric structures (**3**–**6**) depending on the configuration of the two chiral centres of aurantiamide and aurantiamide acetate are possible. To ascertain the stereochemistry of the compounds, **3** and **5** (R = H, COMe) were synthesized starting from optically pure *S* and *R*-*N*-benzoyl phenylalanine which were separately condensed with *S*-phenylalaninol in the presence of *N*,*N'*-dicyclohexylcarbodiimide (DCC) in THF at low temperature. *S*-Phenylalaninol was obtained by the $LiAlH_4$ reduction of *S*-methyl phenylalaninate.

Compound **3a** (R = H) obtained from *S*-*N*-benzoylphenylalanine was found to be identical with natural aurantiamide in all respects (mp, mmp, co-TLC, superimposable IR and ^{1}H NMR spectra). The acetylated product of synthetic **3b** (R = $COCH_3$) was identical with natural aurantiamide acetate. Thus aurantiamide is *N*-(*N'*-benzoyl-*S*-phenylalaninyl)-*S*-phenylalaninol and PA-A the corresponding *O*-acetate.

The diastereoisomer of aurantiamide, viz. dia-aurantiamide (**6a**, R = H) was also synthesized starting from *R*-*N*-benzoylphenylalanine. It was acetylated to give dia-aurantiamide acetate **6b**. The physical and spectral (IR and ^{1}H NMR) properties of aurantiamide and aurantiamide acetate were found to be different from those of the corresponding diastereoisomeric compounds (see Experimental). The amide protons of the diastereoisomeric series resonated at a slightly higher upfield value. A significant difference was observed in the splitting pattern of the methylene protons of the natural and dia-compounds due to different spatial arrangements of these protons in a somewhat rigid framework of the molecules, presumably arising from intramolecular hydrogen bonding or dipolar interactions. In dia-aurantiamide both the benzylic proton-pairs were found to be magnetically equivalent, whereas the third methylene proton-pair were magnetically non-equivalent. In dia-aurantiamide acetate, however, all the methylene protons became magnetically non-equivalent.



3a R = H
3b R = COMe



6a R = H
6b R = COMe

4 Optical antipode of **3**
5 Optical antipode of **6**

All four compounds, viz. aurantiamide, aurantiamide acetate and their diastereoisomers exhibited different ^{1}H NMR spectra in d_6-DMSO from those observed in $CDCl_3$ (see Experimental). Since DMSO can form strong hydrogen bonds with –NH– and –OH groups, intramolecular hydrogen-bonding present in the molecules in $CDCl_3$ will be minimized in the former solvent. Moreover, the spatial arrangements of the different groups in the compounds are also expected to be affected due to dipolar interactions between the compounds and the solvent DMSO. A significant difference in the chemical shift values of the protons in d_6-DMSO from those in $CDCl_3$ has been observed in the case of the amide and OH protons. In $CDCl_3$ the amide protons appeared at *ca* δ 6.8 and *ca* δ 6.3, whereas in d_6-DMSO the corresponding signals were observed at *ca* δ 7.0 and *ca* δ 8.4. The signals for the OH protons were also shifted downfield from *ca* δ 3.2–3.4 in $CDCl_3$ to δ 4.5–4.7 in d_6-DMSO. This large downfield shift is expected as hydrogen-bonding is possible between the amide and OH protons with the >S=O group of the solvent.

The ^{13}C NMR of all four compounds also agreed well with their structures. The assignment could be made on the basis of multiplicity in the SFORD spectra and chemical shift correlations. It is interesting to note that the carbon chemical shifts of all the aliphatic carbons are virtually the same for aurantiamide (**3a**) and its diastereoisomer (**6a**).

EXPERIMENTAL

Mps are uncorr. UV absorption spectra were recorded in 95% aldehyde-free EtOH and IR spectra in Nujol mulls as well as in KBr discs. ^1H NMR spectra were recorded at 100 and 80 MHz in $CDCl_3$ and d_6-DMSO. ^{13}C NMR spectra were recorded at 20 MHz. The $[\alpha]_D$ values reported earlier by us [9] were determined for us at the Department of Chemistry, University of Kalyani. When the rotations were checked later in our Department, the earlier values were found to be incorrect. The values determined at our Department are reported here.

Analytical samples were routinely dried over P_2O_5 *in vacuo* at room temp. or at 80°. Na_2SO_4 was normally used for drying organic solvents. Si gel was used for column chromatography and Si gel G for TLC. Unless otherwise stated, spots were detected with I_2 vapour.

Plant material. Seeds of *P. aurantiacum* Wall. were collected from Assam. A voucher specimen (No PA-S) has been preserved in our laboratory.

Isolation of compounds. Air-dried, coarsely powdered seeds (6-kg batches) were Soxhlet-extracted with petrol for 40 hr. The petrol extract was concd and chromatographed. The combined C_6H_6–$CHCl_3$ (1:3) and $CHCl_3$ eluates on rechromatography and prep. TLC yielded aurantiamide acetate as fine white needles (yield 120 mg; 0.002%), $C_{27}H_{28}N_2O_4$, mp 188° (C_6H_6-cyclohexane), $[\alpha]_D^{30}$ −43° ($CHCl_3$) (Found: C, 72.80, H, 6.40, N, 6.06%; $C_{27}H_{28}N_2O_4$ requires C, 72.95; H, 6.35; N, 6.30%). ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1725 and 1260 (OCOMe), 1660 and 1630 (—CO–N<), 1602, 1580, 745 and 695 (monosubstituted benzene). ^1H NMR (100 MHz, $CDCl_3$): δ 7.72 (2H, *d* of *d*, $J_o = 8$ Hz, $J_m = 2$ Hz, H_o); *ca* 7.46 (3H, *m*, H_m, H_p); 7.25 and 7.12 (5H each, *br s*, *b*,*b'*); 6.74 (1H, *d*, $J = 7.5$ Hz, *c*), disappeared on deuteration; 6.05 (1H, *d*, $J = 8.5$ Hz, *d*), disappeared on deuteration; 4.78 (1H, *m*, *e*); 4.20–4.47 (1H, *m*, *f*); 3.94 (1H, *d* of *d*, $J_{if} = 4.5$ Hz, $J_{ii'} = 11$ Hz, *i*); 3.84 (1H, *d* of *d*, $J_{i'f} = 4.5$ Hz, $J_{i'i} = 11$ Hz, *i'*); 3.22 (1H, *d* of *d*, $J_{ge} = 6$ Hz, $J_{gg'} = 13.5$ Hz, *g*); 3.07 (1H, *d* of *d*, $J_{g'e} = 8$ Hz, $J_{g'g} = 13.5$ Hz, *g'*); 2.74 (2H, *d*, $J = 7$ Hz, *h*,*h'*); 1.98 (3H, *s*, *j*). ^1H NMR (100 MHz, d_6-DMSO): δ 8.42 (1H, *d*, $J = 8.5$ Hz, *c*), disappeared on deuteration, collapsed to singlet on irradiation at δ 4.68; 8.05 (1H, *d*, $J = 8$ Hz, *d*), disappeared on deuteration, collapsed to singlet on irradiation at ~δ 4.0; 7.80 (2H, *d* of *d*, $J_o = 7$ Hz, $J_m = 2.5$ Hz, H_o); 7.40–7.58 (3H, poorly resolved *m*, H_m, H_p); 7.11 (10H, *s*, *b*, *b'*); 4.68 (1H, *d* of *t*, $J_{ec} = 8.5$ Hz, $J_{eg} = 7$ Hz, *e*); 3.74–4.32 (3H, *m*, *f*, *i*); 2.98 (2H, *d*, $J = 7$ Hz, *g*,*g'*), collapsed to singlet on irradiation at δ 4.68; 2.78 (2H, *d*, $J = 6.8$ Hz, *h*,*h'*), collapsed to singlet on irradiation at δ 4.03; 1.96 (3H, *s*, *j*). MS: *m/z* 444 (M^+, 3%), 384 (12, $M^+ - MeCO_2H$), 353 (4, $M^+ - C_6H_5CH_2$), 293 (14, $M^+ - MeCO_2H - C_6H_5CH_2$), 252 [32, $C_6H_5CONH-CH(CO)CH_2C_6H_5$], 224 (17, $C_6H_5CONH-\overset{+}{C}H-CH_2Ph$), 176 (5, $C_6H_5CH_2-CH=CH-\overset{+}{O}R$), 131 (6, $C_6H_5\overset{+}{C}H=CH-CO$), 120 (4, $C_6H_5CH=\overset{+}{N}H_2$), 105 (100, $C_6H_5\overset{+}{C}O$), 91 (15, $C_7\overset{+}{H}_7$), 77 (22, $C_6\overset{+}{H}_5$).

The $CHCl_3$ and $CHCl_3$–EtOH (9:1) eluates on rechromatography afforded aurantiamide, as a white amorphous solid, mp 184° (C_6H_6), $[\alpha]_D^{30}$ −49.5° ($CHCl_3$); ν_{max}^{KBr} cm^{-1}: 3280 (NH, OH), 1650 and 1625 (–CO–N–), 1597, 1573, 755 and 695 (monosubstituted benzene); ^1H NMR (80 MHz, $CDCl_3$): δ 7.60 (2H, *d* of *d*, $J_o = 7$ Hz, $J_m = 2$ Hz, H_o); 7.28–7.41 (3H, *m*, H_m, H_p); 7.16 and 7.02 (5H each, *br s*, *b*,*b'*); 6.70 (1H, *d*, $J = 7$ Hz, *c*), disappeared on deuteration; 5.93 (1H, *d*, $J = 8$ Hz, *d*), disappeared on deuteration; 4.68 (1H, apparent *d* of *t*, $J \approx 8$ Hz, 6.5 Hz, *e*); 3.70–4.20 (1H, *m*, *f*); 3.20–3.45 (3H, *br m*, *i*, *i'*, *j*), changed on deuteration to a 2H, *d* at 3.34 ($J = 5$ Hz, *i*, *i'*); 3.18 (1H, *d* of *d*, $J_{ge} = 6.5$ Hz, $J_{gg'} = 14$ Hz, *g*); 2.94 (1H, *d* of *d*, $J_{g'e} = 8.5$ Hz, $J_{g'g} = 14$ Hz, *g'*); 2.65 (2H, *d*, $J = 7$ Hz, *h*,*h'*). ^1H NMR (100 MHz, d_6-DMSO): δ 8.43 (1H, *d*, $J = 9$ Hz, *c*) disappeared on deuteration; *ca* 7.70–7.90 (1H, *d*, *d*), superimposed on aromatic protons at δ 7.77, disappeared on deuteration; 7.77 (2H, *d* of *d*, $J_o = 7.5$ Hz, $J_m = 2$ Hz, H_o), superimposed on signal for *d*; 7.45–7.60 (3H, *br m*, H_m and H_p); 7.20 (10H, *br s*, *b*, *b'*); 4.70 (1H, *m*, *e*) superimposed on *j*, after D_2O exchange changed to *d* of *d*, $J_{eg} = 5$ Hz, $J_{eg'} = 8.5$ Hz; 4.56–4.86 (1H, *m*, *j*), disappeared on deuteration, partially superimposed on *e*; 3.66–4.06 (1H, *m*, *f*), 2.91–3.20 (2H, *m*, g,g'); *ca* 2.85 (1H, *m*, $J_{hf} = 5$ Hz, $J_{hh'}$ obscured, *h*) and *ca* 2.70 (1H, *m*, $J_{h'f} = 9$ Hz, $J_{hh'}$ obscured, *h'*); 3.37 (2H, *d*, $J = 5$ Hz, *i*). MS (high resolution): *m/z* 402.1890 (M^+ $C_{25}H_{26}N_2O_3$, 0.3%), 384.1838 ($C_{25}H_{24}N_2O_2$, 2.6), 372.1829 ($C_{24}H_{24}N_2O_2$, 2.5), 311.1354 ($C_{18}H_{19}N_2O_3$, 2.7), 293.1282 ($C_{18}H_{17}N_2O_2$, 3.7), 252.1009 ($C_{16}H_{14}NO_2$, 38.5), 224.1070 ($C_{15}H_{14}NO$, 28.6), 134.0662 ($C_9H_{10}O$, 3.5), 131.0494 (C_9H_7O, 6.2), 120.0809 ($C_8H_{10}N$, 10.9), 105.0336 (C_7H_5O, 100), 91.0543 (C_7H_7, 13.6), 77.0376 (C_6H_5, 24.4).

Mild alkaline hydrolysis of PA-A (aurantiamide acetate). A MeOH soln of PA-A (20 mg in 15 ml) was treated with an excess of $NaBH_4$ and kept overnight at room temp. It was worked up in the usual way and extracted with $CHCl_3$. Crystallization from C_6H_6 yielded aurantiamide (17 mg, 94%), mp 184°, $C_{25}H_{26}N_2O_3$ (Found: C, 74.48; H, 6.45; N, 6.80; M^+ 402; $C_{25}H_{26}N_2O_3$ requires C, 74.60; H, 6.51; N, 6.96; MW 402). This compound was identical with natural aurantiamide (mmp, co-TLC, IR, NMR and MS). Hydrolysis of PA-A (5 mg) with 5% NaOH in MeOH (5 ml) at room temp. for 12 hr also furnished aurantiamide, mp 180° (4 mg, 85%).

Acetylation of aurantiamide. A mixture of aurantiamide (10 mg), Ac_2O (0.5 ml) and pyridine (0.1 ml) was shaken for 30 min, kept for another 30 min and worked-up in the usual way. Aurantiamide acetate, $C_{27}H_{28}N_2O_4$, mp 187°, thus obtained (yield: 8 mg; 73%) was identical with PA-A (Found: C, 72.70; H, 6.10; N, 5.98; M^+ 444; $C_{27}H_{28}N_2O_4$ requires C, 72.95; H, 6.35; N, 6.30, MW 444).

S-phenylalaninol was obtained by the $LiAlH_4$ reduction of methyl *S*-phenylalaninate in THF as pale yellow crystals from ether, mp 92° {lit. 95° [12], 91–93° [13]} $[\alpha]_D^{30}$ −27° ($CHCl_3$) and −24.6° (EtOH) {lit. −22.05° (EtOH) [12], −25.6° (EtOH) [13]}. ^1H NMR (80 MHz, $CDCl_3$): δ 7.13 (5H, *br s*, C_6H_5); 3.49 (1H, *d* of *d*, $J_1 = 6$ Hz, $J_2 = 11.5$ Hz) and 3.36 (1H, *d* of *d*, $J_1 = 4.5$ Hz, $J_2 = 11.5$ Hz, $-CH_2OH$); 2.83–3.18 (1H, *m*, –CH–); 2.65 (1H, *d* of *d*, $J_1 = 8$ Hz, $J_2 = 13$ Hz) and 2.49 (1H, *d* of *d*, $J_1 = 5$ Hz, $J_2 = 13$ Hz, $-CH_2Ph$); 2.17 (1H, *br s*, –OH), disappeared on deuteration.

Aurantiamide. S-N-Benzoylphenylalanine (530 mg, 2 mmol) and *S*-phenylalaninol (300 mg, 2 mmol) were dissolved in dry THF (25 ml), cooled to 0° and DCC (600 mg, 3 mmol) in THF (12 ml) added dropwise to the stirred soln. Stirring was continued for 4 hr at 0° and 2 hr at room temp. A few drops of HOAc were then added, stirred for 30 min and filtered. THF was replaced by

EtOAc and a further amount of di-cyclohexylurea which precipitated was filtered. The soln was successively washed with dil. HCl, H_2O, dil. $NaHCO_3$ soln and H_2O. It is known that during the condensation reaction there is the chance of racemization at the *N*-benzoylphenylalanine moiety. However, the extent of racemization was minimized by using DCC [14] and carrying out the reaction under mild conditions for a short time. However, the aurantiamide (250 mg, 30%) formed was contaminated with a small amount of its diastereoisomer, viz. dia-aurantiamide (50 mg, 6%). These two were separated by column chromatography and prep. TLC. The product (**3a**), mp 194° (C_6H_6), $[\alpha]_D^{30}$ −51° ($CHCl_3$) was identical with naturally occurring aurantiamide (mmp, co-TLC, IR, MS and ^1H NMR) (Found: C, 74.30; H, 6.45; N, 6.92; M^+ 402; $C_{25}H_{26}N_2O_3$ requires C, 74.60; H, 6.51; N, 6.96; MW 402). When the coupling was done at higher temperatures, with a longer reaction time (24 hr) a greater degree of racemization was observed.

Dia-aurantiamide. The above procedure was followed by adding DCC (103 mg, ~0.5 mmol) in CH_2Cl_2 (3 ml) to a soln of *R-N*-benzoylphenylalanine (135 mg, 0.5 mmol) and *S*-phenylalaninol (75 mg, 0.5 mmol) in dry CH_2Cl_2 (6 ml). In this case also a small amount of aurantiamide (15 mg, 8%) was formed along with dia-aurantiamide (60 mg, 30%). Dia-aurantiamide, mp 175°, $[\alpha]_D^{30}$ −14.5° ($CHCl_3$) crystallized from C_6H_6–cyclohexane as fine needles. (Found: C, 74.46; H, 6.49; N, 6.97; M^+ 402; $C_{25}H_{26}N_2O_3$ requires C, 74.60; H, 6.51; N, 6.96; MW 402). ν_{max}^{KBr} cm^{-1}: 3440 (–OH), 3300 (–NH), 1655 and 1625 (–CONH), 1595, 1570, 742 and 692 (monosubstituted benzene). ^1H NMR (80 MHz, $CDCl_3$): δ 7.58 (2 H, *d* of *d*, $J_o = 7.5$ Hz, $J_m = 2$ Hz, H_o); 7.24–7.38 (3 H, *m*, H_m, H_p); 6.8–7.2 (11 H, *br m*, *b*, *b'*, *c*); 6.54 (1 H, *d*, $J = 8$ Hz, *d*); 4.75 (1 H, *q*, $J = 7$ Hz, *e*); 3.80–4.30 (1 H, *m*, *f*); 3.25–3.70 (3 H, *br m*, *i*,*i'*, *j*) on deuteration changes to two 1 H, *d* of *d* at δ 3.51 ($J_{i\,f} = 5$ Hz, $J_{ii'} = 11.5$ Hz, *i*) and at δ 3.31 ($J_{i'\,f} = 5$ Hz, $J_{i'i} = 11.5$ Hz, *i'*); 2.98 (2 H, *d*, $J = 7$ Hz, *g*,*g'*); 2.61 (2 H, *d*, $J = 7$ Hz, *h*,*h'*). ^1H NMR (100 MHz, d_6-DMSO): δ 8.35 (1 H, *d*, $J = 8$ Hz, *c*), disappeared on deuteration, collapsed to singlet on irradiation of *e*; 7.95 (1 H, *d*, $J = 9$ Hz, *d*), disappeared on deuteration, collapsed to singlet on irradiation of *f*; 7.76 (2 H, *d* of *d*, $J_o = 7.5$ Hz, $J_m = 2$ Hz, H_o), 7.36–7.55 (3 H, *m*, H_m, H_p); *ca* 7.21 (10 H, *br s*, *b*, *b'*); 4.50–4.80 (2 H, *m*, *e*, *j*) changed to δ 4.66 (1 H, *d* of *d*, $J_{eg} = 6.5$ Hz, $J_{eg'} = 9$ Hz, *e*) after deuteration; 3.78–4.11 (1 H, *m*, *f*); 3.40 (2 H, poorly resolved *t*, $J \approx 5$ Hz, *i*) changed to 2 H, *d* ($J = 5$ Hz) after deuteration, further collapsed to singlet on irradiation of *f*; 2.60–3.0 (4 H, complex *m*, *g*, *g'*, *h*, *h'*). ^{13}C NMR (20 MHz, d_6-DMSO): 62.9 (–O–CH_2–), 52.6 and 55.0 (>N–CH–), 37.6 and 36.8 (benzylic –CH_2–), 171.1 and 166.2 (>C=O).

Aurantiamide acetate (PA-A). Synthetic aurantiamide (60 mg) was acetylated by shaking with Ac_2O (5 ml) in the presence of pyridine (0.1 ml) at room temp. for 30 min and keeping for another 30 min. Synthetic aurantiamide acetate (50 mg, 67%), mp 185° (C_6H_6–cyclohexane), $[\alpha]_D^{30}$ −45° ($CHCl_3$) was found to be identical with natural aurantiamide acetate (mmp, co-TLC, IR, ^1H NMR and MS). (Found: C, 72.74; H, 6.29; N, 6.28; M^+ 444; $C_{27}H_{28}N_2O_4$ requires C, 72.95; H, 6.35; N, 6.30; MW 444).

Dia-aurantiamide acetate. Dia-aurantiamide (20 mg) was acetylated with Ac_2O (1 ml) and pyridine (0.1 ml). Usual work-up yielded dia-aurantiamide acetate (18 mg, 88%), mp 170° (C_6H_6–cyclohexane), $[\alpha]_D^{30}$ −7.80° ($CHCl_3$), (Found: C, 72.62; H, 6.30; N, 5.98; M^+ 444, $C_{27}H_{28}N_2O_4$ requires C, 72.95; H, 6.35; N, 6.30; MW 444). ν_{max}^{KBr} cm^{-1}; 3310 (NH), 1656 and 1627 (CONH), 1730, 1235 ($OCOCH_3$), 1598, 1575, 740 and 690 (monosubstituted benzene). ^1H NMR (100 MHz, $CDCl_3$): δ 7.73 (2 H, *d* of *d*, $J_o = 8$ Hz, $J_m = 2$ Hz, H_o): *ca* 7.38 (3 H, *m*, H_m, H_p); 7.0–7.3 (10 H, *br m*, *b*,*b'*); 6.90 (1 H, *d*, $J = 7.5$ Hz, *c*), disappeared on deuteration; 6.34 (1 H, *d*, $J = 8$ Hz, *d*); 4.80 (1 H, *br q*, $J \approx 7$ Hz, *e*), changed on deuteration to *d* of *d* ($J = 6.5$ Hz, 7.5 Hz); 4.13–4.48 (1 H, *m*, *f*'); 3.97 (1 H, *d*, $J = 4$ Hz, *i*); 3.96 (1 H, *d*, $J = 6$ Hz, *i'*); 3.10 (1 H, *d*, $J = 5.5$ Hz, *g*); 3.09 (1 H, *d*, $J = 7.5$ Hz, *g'*); 2.62 (1 H, *d* of *d*, $J_{h\,f} = 7.5$ Hz, $J_{hh'} = 13$ Hz, *h*); 2.91 (1 H, *d* of *d*, $J_{h'f} = 6.5$ Hz, $J_{h'h} = 13$ Hz, *h'*); 1.91 (3 H, *s*, *j*). ^1H NMR (100 MHz, d_6-DMSO): δ 8.37 (1 H, *d*, $J = 8$ Hz, *c*), disappeared on deuteration, collapsed to singlet on irradiation at δ 4.60; 8.09 (1 H, *d*, $J = 8.5$ Hz, *d*), disappeared on deuteration, collapsed to singlet on irradiation at δ 4.20; 7.78 (2 H, *d* of *d*, $J_o = 7$ Hz, $J_m = 3$ Hz, H_o); 7.38–7.54 (3 H, *m*, H_m, H_p); 7.22 (10 H, *br s*, *b*,*b'*); 4.60 (1 H, *d* of *t*, $J_{ei} = 8$ Hz, $J_{eg} = 7$ Hz, *e*) changed to triplet ($J_{eg} = 7$ Hz) on deuteration; 4.10–4.30 (1 H, *m*, *f*); 2.60–2.96 (4 H, poorly resolved *m*, *g*,*g'*, *h*,*h'*); 3.99 (2 H, *d*, $J = 5$ Hz, *i*); 1.97 (3 H, *s*, *j*).

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