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A DIPEPTIDE DERIVATIVE FROM *HYPERICUM JAPONICUM**

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Abstract—The structure of a novel peptide analogue saropeptate, *N*-benzoyl-L-phenylalanyl-L-phenylalaninol acetate, isolated from the whole plant of *H. japonicum* was elucidated by spectroscopic analysis and its absolute configuration determined by comparison with four synthetic diastereoisomers.

INTRODUCTION

Hypericum japonicum (*Sarothra japonica*) Thunb. (Di-er Cao) has been used in Chinese herbal medicine for the treatment of some bacterial diseases, infectious hepatitis and tumours [1]. We have reported the isolation of nine antimicrobial phloroglucinol derivatives [2–5] and a lactone [6] from the ether extracts, and a flavanonol glycoside [7] from methanolic extracts of this plant. As part of a continuing study of this plant, structural elucidation and synthesis of an uncommon dipeptide derivative, saropeptate (**1**), is reported here.

RESULT AND DISCUSSION

Dried whole plants were extracted with *n*-hexane. The *n*-hexane extract was flash chromatographed repeatedly on silica gel using a *n*-hexane–ethyl acetate system followed by recrystallization from ethyl acetate to obtain **1**.

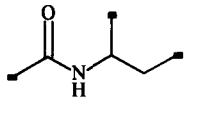
Saropeptate (**1**), colourless needles, showed a molecular ion peak (*m/z*) at 444.2052 for $C_{27}H_{28}O_4N_2$ (calcd; 444.2049) by high resolution mass spectrometry. Its IR spectrum indicated the presence of a ester carbonyl group (1735 cm^{-1}), and amide groups (3410, 1670, 1653 and 1645 cm^{-1}) whose presence were also supported by two proton signals (δ6.73 and 5.93) in the ^1H NMR spectrum and by two amide carbon signals (δ167.11 and 170.25) in the ^{13}C NMR spectrum. The homonuclear correlation 2D COSY (^1H–^1H) experiments indicated the presence of the partial structures of **2** and **3** together with an acetyl group (δ2.02), an unsubstituted benzoyl group at δ7.42–7.70 and two unsubstituted benzyl groups at δ7.05–7.21 and δ7.21–7.30. The presence of these unsubstituted benzoyl and benzyl groups were also confirmed by the intense fragment ions *m/z* 105 (100%) and *m/z* 91 (10%), respectively. The ^{13}C NMR spectrum of **1** gave 27 signals due to two amide carbons, acetyl carbons (δ20.77 and 170.74), 18 aromatic carbons, three methylene carbons (δ37.49, 38.43 and 64.61), two methine carbons (δ49.5 and 55.03). Signal assignments were confirmed by a 2D-long range CH correlation (COLOC) experiment.

The substitution pattern of each group was confirmed by the EI mass spectrum, in which the fragment peaks at *m/z* 224 (21%) and 252 (35%) resulting from bond cleavage at either side of the central carbonyl group was observed. The above spectral data confirmed the structure of **1** as shown.

The absolute configuration of **1** was determined by comparison with four synthetic stereoisomers, *N*-benzoyl-L-Phe-L-phenylalaninol acetate (**1a**), *N*-benzoyl-D-Phe-D-phenylalaninol acetate (**1b**), *N*-benzoyl-D-Phe-L-phenylalaninol acetate (**1c**) and *N*-benzoyl-L-Phe-D-phenylalaninol acetate (**1d**).

The four isomers **1a–d** were synthesized as follows (Scheme 1), respectively. *N-t*-Butyloxy carbazate(Boc)-(L or D)-Phe-*N*-hydroxy-succinimide ester (**4**) was reacted with [*S*-(−);L-form] or [R-(+);D-form]-2-amino-3-phenyl-1-propanol (**5**) in the presence of triethylamine to give the corresponding dipeptide derivative (**6**), which was converted to the acetate (**7**). The treatment of **7** with trifluoroacetic acid to remove the *t*-Boc group and benzoylation of **8** with benzoyl chloride in the presence of triethylamine provided four stereoisomeric derivatives (**1a–d**) whose specific optical rotation values ($CHCl_3$; *c* 1.0) were $[\alpha]_D^{26}$ −40.0° for **1a**, $[\alpha]_D^{26}$ +40.6° for **1b**, $[\alpha]_D^{26}$ −5.0° for **1c** and $[\alpha]_D^{26}$ +4.5° for **1d**. Among those, **1a** showed good agreement with natural saropeptate ($[\alpha]_D^{26}$ −38.8°, $CHCl_3$; *c* 0.041).

The structure **1a** for saropeptate was further supported by HPLC analysis using a Chiralcel OG column, which separated a mixture of **1a–d** as shown in the Experimental. Thus, the retention time of saropeptate was in accord with that of **1a**. Consequently, the structure of saropeptate including its absolute stereochemistry was



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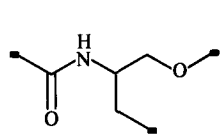

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*Part 7 in the series 'A Flavanonol Rhamnoside from *Hypericum japonicum*'. For part 6 see ref. [7].

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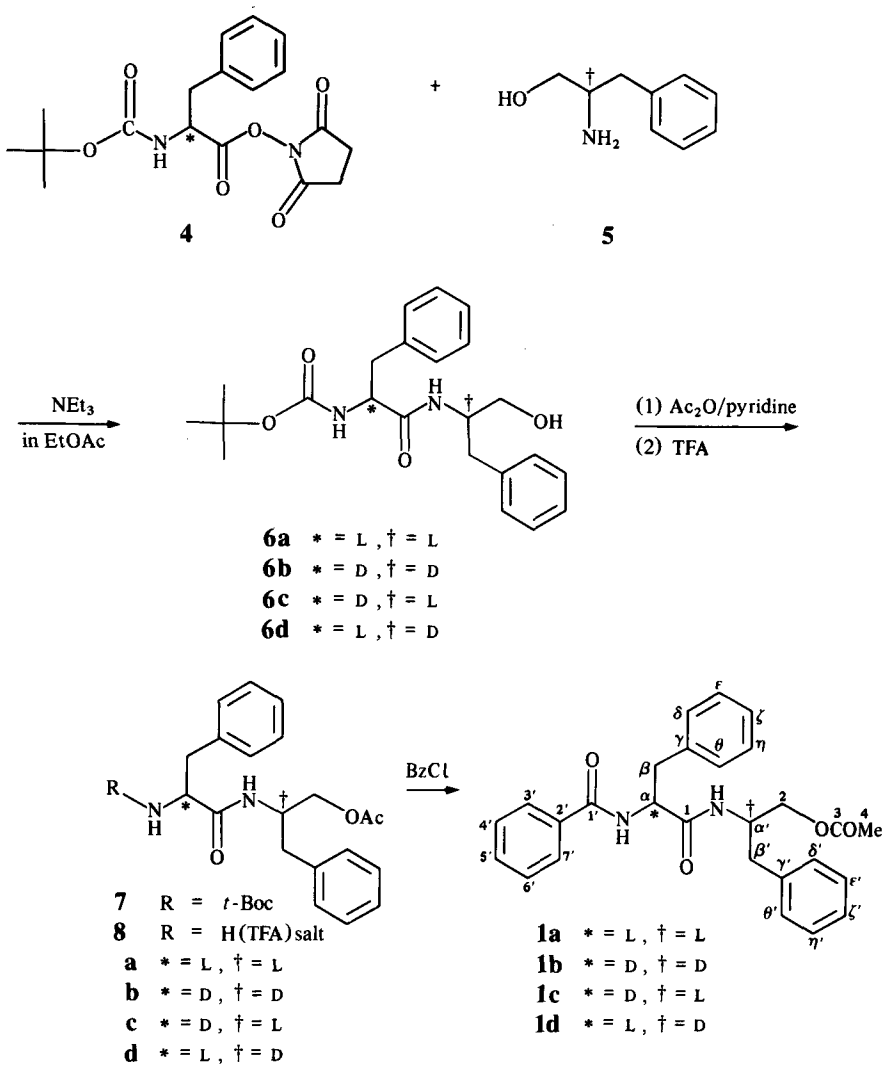

Scheme 1. Synthesis of diastereoisomers of *N*-benzoylphenylalanylphenylalaninol acetate.

determined as *N*-benzoyl-L-Phe-L-phenylalaninol acetate (**1a**).

EXPERIMENTAL

Mps: uncorr.; FAB-MS: operating conditions were primary ion, Xe^+; accelerating voltage, 3 kV (primary) and 3 kV (secondary) and matrix used was *m*-nitrobenzylalcohol (*m*-NBA). HR-MS: the mass range *m/z* 410~460 was scanned at 2.9 sec decade^{-1} (cycle time 60 sec) with a dynamic resolution of 7000.

Plant material. Hypericum japonicum was purchased in Hong Kong, and identified by Dr K. Yoneda (Faculty of Pharmaceutical Sciences, Osaka University). A voucher specimen is kept in our laboratory.

Saropeptate (**1**). Mp 184° (EtOAc), needles, $[\alpha]_D^{26}$ −38.8° ($CHCl_3$; *c* 0.041), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410 (NH), 1735 (CO_2), 1670, 1653 and 1645 (NHC=O), 1500, 1235. UV λ_{max}^{EtOH} nm: 228 (4.08), EI-MS (probe) 70 eV, *m/z* (ret. int.): 43 $[Ac]^+$ (26), 91 $[benzyl]^+$ (10), 105 $[benzoyl]^+$ (100), 224 $[PhCONHCHCH_2Ph]^+$ (21), 252 $[224+CO]^+$ (35), 444 $[M]^+$ (2.5). FAB-MS, *m/z* 77 $[C_6H_5]^+$, 105 $[PhCO]^+$, 289 $[M-2H_2O-PhCONH+1]^+$, 307 $[M-H_2O-PhCONH+1]^+$, 445 $[M+1]^+$. ^{1}H NMR (500 MHz, $CDCl_3$); δ2.02 (3H, s, H-4), 2.75 (2H, *ddd*, $J_{\beta',\beta'}$ = 13.25, $J_{\beta',\alpha'}$ = 7.27 and 6.41, β'-H), 3.07 (1H, *dd*, $J_{\alpha,\beta}$ = 8.55, $J_{\beta,\beta}$ = 13.68, β-H), 3.22 (1H, *dd*, $J_{\alpha,\beta}$ = 5.99, $J_{\beta,\beta}$ = 13.68, β-H), 3.84 (1H, *dd*, $J_{2,\alpha'}$ = 4.27, $J_{2,2}$ = 11.12, H-2), 3.92 (1H, *dd*, $J_{2,\alpha'}$ = 5.13, $J_{2,2}$ = 11.12, H-2), 4.35 (1H, *m*, α'-H), 4.78 (1H, *ddd*, $J_{\alpha,\beta}$ = 5.99 and 8.55, $J_{\alpha,NH}$ = 7.69, α-H), 6.03 (1H, *d*, *J* = 8.55, N'H), 6.73 (1H, *d*, *J* = 7.69, NH), 7.05–7.21 (5H, *m*, δ'~θ'-H), 7.21–7.30 (5H, *m*, δ~θ-H), 7.42 (2H, *t*, *J* = 7.27, H-4', H-6'), 7.51 (1H, *dt*, *J* = 7.27 and 1.21, H-5'), 7.70 (2H, *dd*, *J* = 7.27 and 1.21, H-3', H-7'). ^{13}C NMR (125 MHz, $CDCl_3$); δ20.77 (*q*, C-3), 37.49 (*t*, C-β'), 38.43 (*t*, C-β), 49.51 (*d*, C-α'), 55.03 (*d*, C-α), 64.61 (*t*, C-2), 126.77 (*d*, C-ζ)[a], 127.06 (*d*, C-3', 7'), 127.17 (*d*, C-ζ)[a], 128.61 (*d*, C-4', 6'), 128.65 (*d*, C-ε', η')[b], 128.72 (*d*, C-ε, η)[b], 129.15 (*d*, C-δ', θ')[c], 129.31 (*d*, C-δ, θ)[c], 131.91 (*d*, C-5'), 133.75 (*s*, C-2'), 136.67 (*s*, C-γ')[d], 136.72 (*s*, C-γ)[d], 167.11 (*s*, C-1'), 170.25 (*s*, C-1), 170.74 (*s*, C-3).[a–d] Interchangeable values.

Synthesis of N-t-*BOC*-L-*Phe-phenylalaninol.* (**6**). To *S*(−)-2-amino-3-phenyl-1-propanol(L-form) (**5**) (200 mg, 1.32 mmol) and triethylamine (160 μl) in THF (5 ml), *N*-*t*-BOC-L-Phe-*N*-hydroxy-succinimide ester (**4**) (482 mg, 1.33 mmol) in THF (5 ml) was added dropwisely under cooling in an ice bath for 1 hr and then the resultant soln was stirred overnight at room temp.,

EtOAc was added and the organic layer washed successively with 10% citric acid, 10% $NaHCO_3$ and H_2O and then dried over Na_2SO_4. The solvent was evapd and the crystalline residue recrystallized from EtOH to give crystals **6a** (510 mg, 97%), mp 142°. Calcd for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.58; N, 7.03%. Found: C, 68.96; H, 7.78; N, 6.95%. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410 (NH), 1700 (Boc) and 1670 (NHC=O), 1540, 1490, 1160. FAB-MS, *m/z* 57 $[CMe_3]^+$, 91 $[C_7H_7]^+$, 299 $[M-Me_3OCO]^+$ 399 $[M+1]^+$. **6b**; mp 147°, 84%. **6c**; mp 150°, 96%. **6d**; mp 159°, 94%.

Acetylation of compound **6**. To a soln of **6a** (0.2 g) in pyridine (2 ml) was added Ac_2O (2 ml) and was allowed to stand overnight at room temp. This mixt. was poured into ice-water and the ppt. was collected by filtration to give **7a** as crystals (364 mg, 90%), mp 133°. Calcd for $C_{25}H_{32}N_2O_5$: C, 68.16; H, 7.32; N, 6.36%. Found: C, 67.85; H, 7.45; N, 6.46%. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410 (NH), 1732 (OAc), 1700 (Boc) and 1670 (NHC=O), 1550, 1490, 1235, 1160. FAB-MS *m/z*, 57 $[CMe_3]^+$ (71), 91 $[C_7H_7]^+$, 102 $[CMe_3OCO+1]^+$, 299 $[M-Me_3OCO]^+$, 441 $[M+1]^+$. **7b**; mp 134°, 74%. **7c**; mp 139°, 90%. **7d**; mp 144°, 95%.

Removal of the BOC group from compound **7**. To a soln of **7a** (0.3 g, 0.68 mmol) in CH_2Cl_2 (2 ml) was added dropwise a soln of trifluoroacetic acid (TFA) under cooling in an ice bath during 1 hr and then stirred overnight at room temp. The solvent was evaporated and the crude residue recrystallized from Et_2O to afford crystals **8a** (282 mg, 90%), mp 137°. FAB-MS (*m*-NBA) *m/z*, 43 $[Ac]^+$, 91 $[C_6H_7]^+$, 341 $[M-TFA]^+$. **8b**; mp 138°, 93%. **8c**; oil, 90%. **8d**; oil, 100%.

Benzoylation of compound **8**. Compound **8a**. (250 mg) was dissolved in EtOAc (3 ml), and was neutralized using NEt_3. To this soln was added NEt_3 (55 μl) and benzoyl chloride (77 μl), and this mixt. was stirred for 3 hr at room temp. After addition of H_2O, the organic phase was washed with 10% $NaHCO_3$ and H_2O and then dried over Na_2SO_4. The solvent was evapd and the crystalline residue recrystallized from EtOAc to afford needles **1a** (225 mg, 92%). $[\alpha]_D^{26}$ −40.0° ($CHCl_3$; *c* 1.0), mp 184°. HR-MS: found 444.2058, $C_{27}H_{28}N_2O_4$ requires 444.2049. Anal. calcd for $C_{27}H_{28}N_2O_4 \cdot 1/4\,H_2O$: C, 72.22; H, 6.31; N, 6.23%. Found: C, 72.45; H, 6.26; N, 6.25%. FAB-MS *m/z* 77 $[C_6H_5]^+$, 105 $[PhCO]^+$, 289 $[M-2H_2O-PhCONH+1]^+$, 307 $[M-H_2O-PhNH+1]^+$, 445 $[M+1]^+$ This compound was identical with natural saropeptate by comparison of TLC and spectral data.

Isomers **1b–d** were prepd by the same method as described for **1a**. **1b**: mp 185°, 83%, $[\alpha]_D^{26}$ +40.6° ($CHCl_3$; *c* 1.0). IR, ^1H and ^{13}C NMR spectrum were identical with **1a**. **1c**: mp 175°, 75%, $[\alpha]_D^{26}$ −5.0° ($CHCl_3$; *c* 1.0). IR was identical with **1a** and **b**. ^1H NMR (500 MHz, $CDCl_3$); δ1.96 (3H, *s*, H-4), 2.61 (1H, *dd*, $J_{\beta',\beta'}$ = 13.62, $J_{\beta',\alpha'}$ = 8.12, β'-H), 2.78 (1H, *dd*, $J_{\beta',\beta'}$ = 13.62, $J_{\beta',\alpha'}$ = 5.99, β'-H), 3.07 (1H, *dd*, $J_{\alpha,\beta}$ = 8.12, $J_{\beta,\beta}$ = 13.67, β-H), 3.22 (1H, *dd*, $J_{\alpha,\beta}$ = 5.98, $J_{\beta,\beta}$ = 13.67, β-H), 3.94 (1H, *dd*, $J_{2,\alpha'}$ = 5.55, $J_{2,2}$ = 11.54, H-2), 3.98 (1H, *dd*, $J_{2,\alpha'}$ = 4.27, $J_{2,2}$ = 11.54, H-2), 4.34 (1H, *m*, α'-H), 4.79 (1H, *ddd*, $J_{\alpha,\beta}$ = 5.99 and 8.12, $J_{\alpha,NH}$ = 7.26, α-H), 6.03 (1H, *d*, *J* = 8.12, N'H), 6.74 (1H, *d*, *J* = 7.26, NH), 7.08–7.23 (5H, *m*, $\delta' \sim \theta'$-H), 7.24–7.32 (5H, *m*, $\delta \sim \theta$-H), 7.42 (2H, *t*, *J* = 7.69, 4', 6'-H), 7.51 (1H, *dt*, *J* = 7.69 and 1.28, 5'-H), 7.70 (2H, *dd*, *J* = 7.69 and 1.28, 3', H-7'). **1d**: mp 175°, 91%, $[\alpha]_D^{24}$ +4.8° ($CHCl_3$; *c* 1.0). IR and ^1H NMR spectrum were identical with **1c**.

Analysis by HPLC. Chiralcel OG column (Daicel Chemical Ind.), 25 × 0.46 cm i.d. was employed for the separation of the four isomers **1a–d** using *n*-hexane–EtOH (19:1) at a flow rate of 1.2 ml min^{-1} with detection at 254 nm. Compounds **1a–d** had R_ts at 13.78, 19.12, 24.50 and 29.45 min respectively. Natural saropeptate was eluted at 13.40 min.

REFERENCES

1. Chang Su New Medical College (1977) *Dictionary of Chinese Crude Drugs*. Shanghai Scientific Technological Publishers, Shanghai.
2. Ishiguro, K., Yamaki, M. and Takagi, S. (1985) *J. Chem. Soc. Chem. Commun.* 26.
3. Ishiguro, K., Yamaki, M., Kashihara, M. and Takagi, S. (1986) *Planta Med.* 247.
4. Ishiguro, K., Yamaki, M., Kashihara, M. and Takagi, S. (1986) *Planta Med.* 395.
5. Ishiguro, K., Yamaki, M., Kashihara, M., Takagi, S. and Koichiro Isoi (1990) *Phytochemistry* **29**, 1010.
6. Ishiguro, K., Yamaki, M., Kashihara, M., Takagi, S. and Koichiro Isoi (1990) *Planta Med.* **56**, 274.
7. Ishiguro, K., Nagata, S., Fukumoto, H., Yamaki, M., Takagi, S. and Koichiro Isoi (1991) *Phytochemistry* **30** (in press).