Dimorphamides A – C, New Polyphenolic Amides from *Atriplex dimorphostagia*

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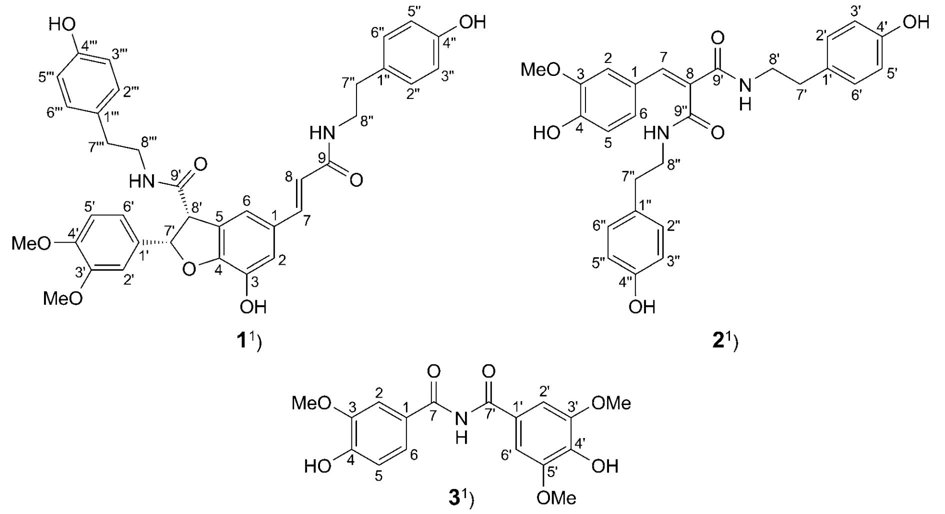
Dimorphamides A – C (**1** – **3**, resp.), three new polyphenolic amides, have been isolated from the BuOH-soluble fraction of the whole plant of *Atriplex dimorphostagia*, along with two known compounds, thymidine and *N*-[(*E*)-feruloyl]tyramine. Their structures have been determined on the basis of spectral studies.

Introduction. – *Atriplex* is an important genus of the family Chenopodiaceae which comprises *ca.* 200 species. In Pakistan, it is represented by eleven species. One of these is *Atriplex dimorphostagia*, which occurs in Southern Asia and North Africa. It commonly grows in Pakistan in Karachi, Quetta, and Chaman [1]. The plants of the genus *Atriplex* are used as fungicide and for treatment of bronchitis [2]. Literature survey revealed that no phytochemical and pharmacological studies have so far been carried out on *Atriplex dimorphostagia*. This prompted us to carry out phytochemical studies on this species which resulted in the isolation and structure elucidation of three new amides named dimorphamides A – C (**1** – **3**, resp.), in addition to thymidine [3] and *N*-*trans*-feruloyltyramine [4].

Results and Discussion. – 1. *Chemistry.* The EtOH extract of the whole plant of *A. dimorphostagia* was processed as described in the *Exper. Part* to afford three new polyphenolic amides, dimorphamides A – C (**1** – **3**, resp.), along with two known compounds for the first time from the species (*Fig. 1*). The compounds **1** – **3** showed positive ferric chloride test for phenols.

Dimorphamide A (**1**) was obtained as a white amorphous solid. The IR spectrum showed the presence of OH (3400 – 3450 cm^{-1}) and NH groups (3340 – 3390 cm^{-1}), and amide C=O (1654 cm^{-1}) and conjugated C=C (1610 cm^{-1}) functionalities. The UV spectrum showed absorption maxima at 223, 287, and 319 nm. The HR-FAB-MS showed a *pseudo*-molecular $[M+H]^+$ peak at *m/z* 625.2549, consistent with the molecular formula $C_{36}H_{37}N_2O_8$ (calc. 625.2554) indicating 20 degrees of unsaturation. The molecular formula was supported by ^{13}C-NMR spectra, broad-band (BB) and distortionless enhancement by polarization transfer (DEPT), comprised signals for two Me, four CH_2, and seventeen CH groups, and 13 quaternary C-atoms (*Table 1*). The downfield signals at $\delta(C)$ 172.9 and 169.0 could be assigned to two amide CO groups, while the signals of two MeO groups were observed at $\delta(C)$ 56.7 and 56.3, respectively. The olefinic C-atoms resonated at $\delta(C)$ 141.7 and 119.4. The data were



1¹)　**2**¹)　**3**¹)

Fig. 1. *Structures of Compounds* **1**–**3**

closely related to those of the known lignanamides, namely grossamide [5] and thoreliamide [5]. The ^{1}H-NMR spectrum (*Table 1*), combined with the ^{1}H,^{1}H-COSY spectrum displayed signals for two tyramine moieties (two NCH_2CH_2 groups: δ(H) 2.71 (*t*, *J* = 7.3, 2 H), 3.41 (*t*, *J* = 7.3, 2 H), 2.75 (*t*, *J* = 7.1, 2 H), 3.45 (*t*, *J* = 7.1, 2 H)), two *para*-substituted phenyl rings (δ(H) 7.02 (*d*, *J* = 8.4, 2 H), 6.99 (*d*, *J* = 8.4, 2 H), 7.03 (*d*, *J* = 8.4, 2 H), 6.72 (*d*, *J* = 8.4, 2 H)), two MeO groups at δ(H) 3.87 and 3.80, one (*E*)-C=C bond (δ(C) 7.39 (*d*, *J* = 15.9, 1 H), 6.40 (*d*, *J* = 15.9, 1 H)), and characteristic signals of a benzofuran type lignan (δ(H) 5.86 (*d*, *J* = 8.4, 1 H), 4.15 (*d*, *J* = 8.4, 1 H)). HMBC Correlations CH_2(8″)/C(9) and CH_2(8‴)/C(9′) suggested the attachment of the two tyramine moieties to C(9) and C(9′) *via* amide bonds, respectively (*Fig. 2*). The presence of two tyramine units was also confirmed by EI-MS which displayed peaks of the fragment ions $[M - \text{tyr}]^+$, $[M - 2\text{tyr}]^+$, $[M - 2\text{tyr} - \text{ring } B]^+$, and $[\text{OH–}C_6H_4\text{–}CH_2\text{–}CH_2\text{–}NH_2]^+$ (*m*/*z* 137). All of these data were similar to those of grossamide and threseliamide. The chemical shifts and coupling constants of the aromatic rings showed closer agreement with those of grossamide, revealing the presence of a 1,3,4-trisubstituted and a 1,3,4,5-tetrasubstituted phenyl group each in the molecule. The compound **1** is, therefore, a positional isomer of grossamide. The peak of the fragment ion $[M - 2\text{ tyramine} - \text{ring } B]^+$ in the EI-MS was observed at *m*/*z* 215 in contrast to a similar fragment-ion peak observed in grossamide at *m*/*z* 229 [5], suggesting that trisubstituted phenyl ring contains two MeO groups. It could be confirmed by HMBC and NOE interactions. The MeO signals at δ(H) 3.87 and 3.80 showed ^3J correlations with those of C(3′)¹) and C(4′), respectively. Irradiation of MeO H-atoms at δ(H) 3.87 caused enhancement of the signal of H–C(5′). On the other hand, irradiation at δ(H) 3.80 caused enhancement of signal of H–C(2′). The relative configuration at C(7′) and

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¹) Arbitrary numbering. For systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD) *of Compound* **1**. δ in ppm, *J* in Hz. Arbitrary C-atom numbering as indicated in *Fig. 1*.

Position	δ(H)[a])	δ(C)[b])	Position	δ(H)[a])	δ(C)[b])
1			1″		131.0
2	7.10 (br. *s*)	113.0	2″	6.72 (*d*, *J* = 8.4)	130.9
3		129.4	3″	7.03 (*d*, *J* = 8.4)	116.2
4		130.4	4″		156.9
5		151.1	5″	7.03 (*d*, *J* = 8.4)	116.2
6	6.73 (br. *s*)	118.0	6″	6.72 (*d*, *J* = 8.4)	130.9
7	7.39 (*d*, *J* = 15.9)	141.7	7″	2.75 (*t*, *J* = 7.1)	35.7
8	6.40 (*d*, *J* = 15.9)	119.4	8″	3.45 (*t*, *J* = 7.1)	42.2
9		169.0	1‴		131.2
1′		132.5	2‴	6.99 (*d*, *J* = 8.4)	130.7
2′	6.90 (*d*, *J* = 1.5)	110.4	3‴	7.02 (*d*, *J* = 8.4)	116.4
3′		148.0	4‴		156.9
4′		146.0	5‴	7.02 (*d*, *J* = 8.4)	116.4
5′	6.69 (*d*, *J* = 8.4)	116.3	6‴	6.99 (*d*, *J* = 8.4)	130.7
6′	6.76 (*dd*, *J* = 1.5, 8.4)	120.0	7‴	2.71 (*t*, *J* = 7.3)	35.3
7′	5.86 (*d*, *J* = 8.4)	89.9	8‴	3.41 (*t*, *J* = 7.3)	42.6
8′	4.15 (*d*, *J* = 8.4)	58.6	*Me*O–C(3′)	3.80 (*s*)	56.3
9′		172.9	*Me*O–C(4′)	3.87 (*s*)	56.7

[a]) Recorded at 300 MHz. [b]) Recorded at 125 MHz.

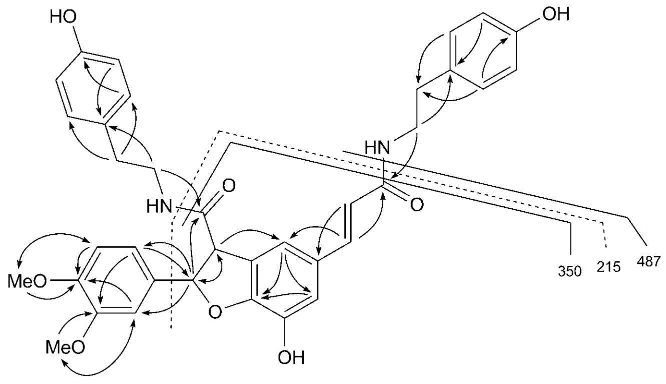

Fig. 2. *Key HMBC* (H → C) *and NOE* (↔) *correlations, and MS fragmentations of* **1**

C(8′) was established as *cis* on the basis of the coupling constant (8.4 Hz) for H–C(7′) and H–C(8′), and further confirmed by NOESY correlations between both these H-atoms. The optical inactivity suggested that it was racemic. The structure of dimorphamide is, therefore, assigned as shown in *Fig. 1*.

Dimorphamide B (**2**) was obtained as a colorless gummy solid. The UV spectrum was characteristic of amides of hydroxycinnamic acids. The IR spectrum showed bands for OH (3450 cm^{-1}) and NH groups (3340–3390 cm^{-1}), and amide C=O (1655 cm^{-1}), and conjugated C=C functions (1610 cm^{-1}). The HR-FAB-MS (negative-ion mode) showed a *pseudo*-molecular $[M-\mathrm{H}]^-$ peak at *m/z* 475.1869, consistent with molecular formula $C_{27}H_{27}N_2O_6$ (calc. 475.1873). The ^{13}C-NMR (BB and DEPT) exhibited 27 signals including those for one Me group, four CH_2 and twelve CH groups, and ten quaternary C-atoms (see *Table 2*). The downfield signal at δ(C) 167.9 was due to amide CO group, while signals for olefinic C-atoms were observed at δ(C) 142.1 and 127.9. The O-bearing aromatic C-atoms gave signals at δ(C) 156.8, 150.1, and 149.0. The ^{1}H-NMR (*Table 2*), combined with ^{1}H,^{1}H-COSY spectrum, displayed the signals of tyramine moiety (NCH_2CH_2 group at 2.30 (*t*, *J* = 7.0, 2 H), 3.12 (*t*, *J* = 7.0, 2 H); *para*-substituted phenyl ring (6.58 (*d*, *J* = 8.7, 1 H), 6.81 (*d*, *J* = 8.7, 1 H)), one MeO group (3.73), one trisubstituted conjugated C=C bond H-atom (7.82 (*s*, 1 H)), and characteristic signals of a 1,3,4-trisubstituted phenyl ring (6.75 (*d*, *J* = 8.4, 1 H), 7.00 (*dd*, *J* = 1.5, 8.4, 1 H), and 7.16 (*d*, *J* = 1.5, 1 H)). All these data closely resemble to those of *N*-[(*E*)-

Table 2. *^{1}H- and ^{13}C-NMR Data* (CD_3OD) *of Compounds* **2** *and* **3**. δ in ppm, *J* in Hz. Arbitrary C-atom numbering as indicated in *Fig. 1*.

Position	**2**		**3**	
	δ(H)[a]	δ(C)[b]	δ(H)[a]	δ(C)[b]
1		127.2		123.1
2	7.16 (*d*, *J* = 1.5)	113.4	7.55 (*d*, *J* = 2.0)	113.8
3		149.0		148.6
4		150.1		152.6
5	6.75 (*d*, *J* = 8.4)	116.4	6.81 (*d*, *J* = 8.5)	115.8
6	7.00 (*dd*, *J* = 1.5, 8.4)	126.3	7.53 (*dd*, *J* = 2.0, 8.5)	125.2
7	7.82 (*s*)	142.1		170.0
8		127.9		
1′		131.0		122.0
2′	6.81 (*d*, *J* = 8.4)	130.6	7.32 (*s*)	108.3
3′	6.58 (*d*, *J* = 8.4)	116.2		148.8
4′		156.8		141.8
5′	6.58 (*d*, *J* = 8.4)	116.2		148.8
6′	6.81 (*d*, *J* = 8.4)	130.6	7.32 (*s*)	108.3
7′	2.30 (*t*, *J* = 7.0)	35.4		169.9
8′	3.12 (*t*, *J* = 7.0)	42.8		
9′		167.9		
1″		131.0		
2″	6.81 (*d*, *J* = 8.4)	130.6		
3″	6.58 (*d*, *J* = 8.4)	116.2		
4″		156.8		
5″	6.58 (*d*, *J* = 8.4)	116.2		
6″	6.81 (*d*, *J* = 8.4)	130.6		
7″	2.30 (*t*, *J* = 7.0)	35.4		
8″	3.12 (*t*, *J* = 7.0)	42.8		
9″		167.9		
*Me*O–C(3)	3.73 (*s*)	56.2	3.88 (*s*)	56.4
*Me*O–C(3′)			3.87 (*s*)	56.8
*Me*O–C(5′)			3.87 (*s*)	56.8

[a] Recorded at 300 MHz. [b] Recorded at 125 MHz.

feruloyl]tyramine. The major difference was found in the olefinic region, the latter compound possessing a disubstituted C=C bond. The compound **2** has an additional substituent attached to the non-conjugated olefinic C-atom. The intense ^{13}C-NMR signals of tyramine moiety revealed the presence of a second tyramine unit which was also confirmed by EI-MS showing peaks for fragment ions $[M - \text{tyr}]^+$ at m/z 339, $[M - 2\ \text{tyr}]^+$ at m/z 202, and $[NH_2–CH_2–CH_2–C_6H_4–OH]^+$ at m/z 137. The HMBC correlations $CH_2(8')/C(9')$ and $CH_2(8'')/C(9'')$ confirmed that both tyramine units are joined to CO C-atoms *via* amides. On the basis of these evidences, the structure of dimorphamide **2** was assigned as shown in *Fig. 1*.

Dimorphamide C (**3**) was obtained as a colorless gummy solid. The IR spectrum showed bonds for OH (3450 cm^{-1}) and chelated NH groups (3260 cm^{-1}), and amide C=O function (1654 cm^{-1}). The UV spectrum showed absorption maxima at 217 and 263 nm. The HR-EI-MS showed a molecular-ion peak at m/z 347.1005, consistent with the molecular formula $C_{17}H_{17}NO_7$ (calc. 347.1009). The molecular formula was further supported by ^{13}C-NMR (BB and DEPT) which showed seventeen signals corresponding to three Me, five CH groups, and nine quaternary C-atoms (*Table 2*). The downfield signals at δ(C) 170.0 and 169.9 could be attributed to amide CO groups, while signals of five O-bearing aromatic C-atoms were observed at δ(C) 152.6, 148.8 (2 C), 148.6, and 141.8. The signals at δ(C) 56.8 (2 C) and 56.4 were assigned to MeO groups. The ^{1}H-NMR spectrum in (D_6)DMSO (*Table 2*) showed a downfield signal of a chelated NH group at δ(H) 11.86, but the aromatic H-atom signals were clustered. A better resolved spectrum was obtained in CD_3OD and revealed the presence of a 1,3,4,5-symmetrical tetrasubstituted Ph ring (δ(H) 7.32 (*s*, 2 H)). Both of these H-atoms showed ^3J correlation in HMBC with the amide CO group at δ(C) 169.9. In EI-MS the fragment-ion peaks at m/z 181 and 153 confirmed the presence of two MeO groups and one OH group. A 1,3,4-trisubstituted Ph ring could also be inferred by ^{1}H-NMR (δ(H) 7.55 (*d*, $J = 2.0$, 1 H), 7.53 (*dd*, $J = 8.5, 2.0$, 1 H), and 6.81 (*d*, $J = 8.5$, 1 H)), and further confirmed by a fragment-ion peak at m/z 151 in EI-MS. Both H–C(2) and H–C(6) showed HMBC correlations with amide CO group at δ(C) 170.0. The MeO H-atoms (δ(H) 56.8) showed ^3J correlations with C(3′)[1]) and C(5′). Irradiation of these H-atoms caused enhancement of signals of H–C(2′) and H–C(6′). On the other hand, the MeO H-atoms (δ(H) 56.4) showed ^3J correlation with C(3) at δ(C) 148.6. Irradiation of these H-atoms caused enhancement of the signal of H–C(2) (δ(H) 7.55). The chelated NH H-atom itself showed ^2J correlations with both the amide CO groups in agreement with the assigned structure of dimorphamide C (**3**) as 4-hydroxy-*N*-(4-hydroxy-3-methoxybenzoyl)-3,5-dimethoxybenzamide (*Fig. 1*).

Experimental Part

General. Column chromatography (CC): *Diaion HP-20* (*Nippon Rensui Co.*, Tokyo, Japan) and silica gel (SiO_2, 230–400 mesh; *E. Merck*, D-Darmstadt). TLC: precoated silica gel *60* F_{254} plates (20 × 20 cm, 0.2-mm thick; *E. Merck*, D-Darmstadt); visualization at 254 nm and by spraying with ceric sulfate reagent. HPLC: for final purification on a recycling prep. HPLC (*LC-908W-C-60*, *Japan Analytical Industry Co. Ltd.*, Tokyo, Japan) with a column of *ODS-M-80* (4 μm, 250 × 20 mm, *Japan Analytical Industry Co. Ltd.*, Tokyo, Japan). Optical rotations: *ATAGO AP-300* digital polarimeter using a 200 mm tube. UV and IR spectra: *Hitachi-UV-3200* and *Jasco-320-A* spectrometers, resp. ^{1}H-and ^{13}C-NMR, COSY, HMQC, and HMBC spectra: *Bruker AM-400* spectrometer operating at 400 (^{1}H) and 100 MHz

(^{13}C), resp.; the chemical shifts in ppm (δ), and the coupling constants (J) in Hz. EI-MS and HR-FAB-MS (matrix: glycerol): *JEOL JMS-HX110* and *JMS-DA 5000* mass spectrometers.

Plant Material. The whole plants of *Atriplex dimorphostagia* KAR. et KIR. were collected from Gaddap area of Sindh Province in April 2005 and identified by Dr. *S. Khatoon*, Plant Taxonomist, Department of Botany, University of Karachi, with which a voucher specimen (No. 05 and G. H. No. 78271) has been deposited.

Extraction and Isolation. The whole plants of *Atriplex dimorphostagia* (23 kg) were shade-dried, ground, and extracted with MeOH (3 × 50 l, 10 d each) at r.t. The combined MeOH extract (475.67 g) was successively extracted with hexane (10 l; 140 g), $CHCl_3$, (8 l; 80 g), AcOEt (10 l; 125 g), BuOH (5 l; 60.67 g), and H_2O (3 l; 70 g). The BuOH-soluble fraction (60.67 g) was chromatographed over *Diaion HP-20*, and eluted with mixtures of MeOH/H_2O in decreasing order of polarity to obtain the three major *Fractions A–C*. *Fr. A*, eluted with MeOH/H_2O 50:50 (7.57 g), was chromatographed over SiO_2 and eluted with $CHCl_3$/MeOH in increasing order of polarity to obtain the three *Subfractions* B_A–B_C. *Subfr.* B_B, obtained from $CHCl_3$/MeOH 9.3:0.7 (2.9 g), was again submitted to CC (SiO_2; $CHCl_3$/MeOH 9.6:0.4) to yield 100-ml fractions. *Frs. 4* and *5* were combined (20 mg) and subjected to HPLC (MeOH/H_2O 2:1 at a flow rate of 4 ml/min) to provide *dimorphamide C* (**3**; 13.0 mg; t_R 28 min). *Fr.* B_C (1.7 g), obtained from $CHCl_3$/MeOH 9.1:0.9, was again subjected to CC (SiO_2; $CHCl_3$/MeOH 9.5:0.5) to collect 100-ml fractions. The *Frs. 6* and *7* (15 mg) with similar TLC profiles were combined and subjected to HPLC (MeOH/H_2O 1:1 at a flow rate 3 ml/min) to obtain thymidine (10.5 mg; t_R 21 min). The *Fr. C*, eluted with MeOH/H_2O 75:25 (6.05 g), was subjected to CC (SiO_2; $CHCl_3$/MeOH 9.5:0.4) to collect 100-ml fractions. *Fr. 1* provided *N*-[(*E*)-feruloyl]tyramine (11.6 mg). *Fr. 5* provided a raw compound which was purified by HPLC (MeOH/H_2O 2:1 at a flow rate 3 ml/min) to furnish *dimorphamide* A (**1**; 8.0 mg; t_R 29 min). *Frs. 7* and *8* with similar TLC profiles were combined and subjected to HPLC (MeOH/H_2O 2:1 at a flow rate 3 ml/min) to yield *dimorphamide B* (**2**; 7.05 mg; t_R 30 min).

The structures of the isolated known compounds were elucidated by comparison of their physical and spectral data with those reported in literature [4][5].

Dimorphamide A (*=(*2RS,3SR*)-2-(3,4-Dimethoxyphenyl)-2,3-dihydro-7-hydroxy-*N*-[2-(4-hydroxyphenyl)ethyl]-5-[(*E*)-3-{[2-(4-hydroxyphenyl)ethyl]amino}-3-oxoprop-1-en-1-yl]-1-benzofuran-3-carboxamide*; **1**). White amorphous solid. $[\alpha]_D^{20} = 0$ ($c = 0.05$, MeOH). UV ($CHCl_3$): 223 (4.27), 287 (3.97), 319 (3.96). IR (KBr): 3400–3450, 3340–3390, 1654, 1610, 960. ^{13}C- and ^1H-NMR: see *Table 1*. EI-MS: 487 (100), 350 (50), 215 (53), 137 (45). HR-FAB-MS (pos.): 625.2549 ($[M+H]^+$, $C_{36}H_{37}N_2O_8^+$; calc. 625.2554).

Dimorphamide B (*=2-(4-Hydroxy-3-methoxybenzylidene)-*N,N′*-bis[2-(4-hydroxyphenyl)ethyl]propanediamide*; **2**). Colorless gummy solid. UV ($CHCl_3$): 219 (4.21), 232 (sh, 3.89), 290 (4.20), 321 (3.90). IR (KBr): 3450, 3340–3390, 1655, 1610, 960. ^{13}C- and ^1H-NMR: see *Table 2*. EI-MS: 339 (100), 202 (55), 137 (35). HR-FAB-MS (neg.): 475.1869 ($[M-H]^-$, $C_{27}H_{27}N_2O_6^-$; calc. 475.1873).

Dimorphamide C (*=4-Hydroxy-*N*-(4-hydroxy-3-methoxybenzoyl)-3,5-dimethoxybenzamide*; **3**): Colorless gummy solid. UV ($CHCl_3$): 217 (4.46), 263 (4.83). IR (KBr): 3450, 3260, 1654. ^{13}C- and ^1H-NMR: see *Table 2*. EI-MS: 347 (100), 181 (40), 153 (34), 151 (50). HR-EI-MS: 347.1005 (M^+, $C_{17}H_{17}NO_7^+$; calc. 347.1009).

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