Novel Synthesis of Dianthalexin (Phytoalexin) Analogues* Preparation

M. Hauteville*

Laboratoire de Biochimie Végétale, I. C. B. M. C., Université Claude Bernard-Lyon I,
43 Boulevard du 11 Novembre 1918,
69622 Villeurbanne cedex, France

M. Ponchet and P. Ricci

Station de Botanique et de Pathologie Végétale, INRA, Villa Thuret, BP 78,
Antibes 06602, France

J. Favre-Bonvin

Laboratoire de Mycochimie, I. C. B. M. C., Université Claude Bernard-Lyon I,
43 Boulevard du 11 Novembre 1918,
69622 Villeurbanne cedex, France

Received September 30, 1987

Two phytoalexins, previously isolated from carnation infected by *Phytophthora parasitica* D., were synthesized: 2-phenyl-7-hydroxy-4*H*-3,1-benzoxazin-4-one (Dianthalexin) and 2-(2-hydroxybenzoyl)-amino-4-methoxybenzoic acid (Dianthramide A). The first one was obtained by using potassium *t*-butoxide in dimethylformamide to prevent its heterocycle opening. Five novel other analogous compounds were also prepared.

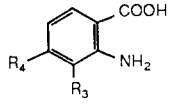
J. Heterocyclic Chem., **25**, 715 (1988).

From carnation's tissues (*Dianthus caryophyllus* L.) infected by *Phytophthora parasitica* D. or elicited by treatment with a mycelial extract of this fungus, three novel compounds were isolated and described as phytoalexins: Dianthalexin (**3a**) [1] with a 4*H*-3,1-benzoxazin-4-one ring and Dianthramides A (**4b**) and B (**4c**) [2] in which this ring is opened.

Only three other phytoalexins of this chemical group were previously found in oat (*Avena sativa*): Avenalumins I, II and III [3,4].

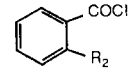
A small sample of Dianthalexin was obtained by thermal treatment of 2-benzoylamino-4-hydroxybenzoic acid (**4a**), but together with its decarboxylation's product and other by-products, resulting in a poor yield [1]. A more efficient method is described here and Dianthramide A and five other novel compounds: **4d, 3f, 4f, 3g** and **4g**, were also synthesized.

In the first stage Bain and Smalley's method [5] was directly leading to the **3** heterocyclic form. Then, by condensation between 4-hydroxyanthranilic acid (**1b**) and benzoyl chloride (**2a**) in excess (in pyridine), the cyclized but also esterified compound $\mathbf{3a_0}$ was obtained. The saponification, necessary to release the 7-hydroxyl group without opening heterocycle, was conducted in dimethylformamide by using potassium *t*-butoxide.



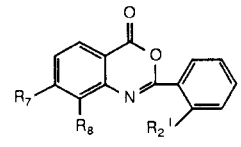
1

1a, $R_3 = R_4 = H$
1b, $R_3 = H$, $R_4 = OH$
1c, $R_3 = H$, $R_4 = OCH_3$
1d, $R_3 = OH$, $R_4 = H$

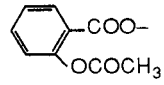
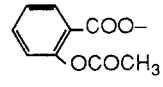


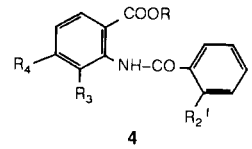
2

2a, $R_2 = H$
2b, $R_2 = OCOCH_3$



3

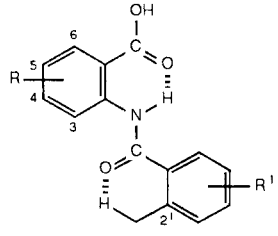
3	R_2'	R_7	R_8	
$3a_o$	-H	$-OCOC_6H_5$	-H	
3a	-H	-OH	-H	Dianthalexin
$3b_o$	$-OCOCH_3$	$-OCH_3$	-H	
$3d_o$	$-OCOCH_3$		-H	
3e	-H		-H	
$3f_o$	-H	$-OCH_3$	$-OCOC_6H_5$	
3f	-H	-H	-OH	
$3g_o$	$-OCOCH_3$	-H		
3g	-OH			
$3h_o$	$-OCOCH_3$	-H	-OH	
		-H	-H	



4

4	R	R_2'	R_3	R_4	
4a	-H	-H	-H	-OH	
4b	-H	-OH	-H	$-OCH_3$	Dianthramide A
4c	$-CH_3$	-OH	-H	-OH	Dianthramide B
4d	-H	-OH	-H	-OH	
4f	-H	-H	-OH	-H	
4g	-H	-OH	-OH	-H	
4h	-H	-OH	-H	-H	

By this way Dianthalexin was prepared with a better yield and the same method was then applied from the four anthranilic acids: **1a, 1b, 1c** or **1d**. Their condensation with appropriate aroyl chloride **2a** or **2b**, always in excess, gave the owing six 4*H*-3,1-benzoxazin-4-ones: $\mathbf{3b_0}$, $\mathbf{3d_0}$, $\mathbf{3h_0}$, $\mathbf{3g_0}$, $\mathbf{3f_0}$ and **3e**. But saponification of compounds $\mathbf{3b_0}$-$\mathbf{g_0}$ in above mentioned conditions nevertheless gave also opened products. A possible explanation is that the liberated 2'-hydroxyl group stabilizes the opened form by hydrogen bond with the *ortho* carbonyl group, **5**.



5

As it appeared difficult to resolve the mixtures of both opened and cyclic forms, these derivatives together with $\mathbf{3f_0}$ were treated by sodium hydroxide, in acetone, to only obtain the opened form.

The five corresponding compounds finally isolated: **4b** (Dianthramide A), **4d, 4h, 4g** and **4f** were then heated to obtain their cyclized homologues. The first three were decomposed and the two others gave effectively **3g** and **3f**.

Isolated compounds were analyzed with uv, ir, ms and in some cases with ^{1}H-nmr. All uv spectra are showing several peaks and shoulders but without characteristic profile for opened or cyclized forms. In contrast, other physical characteristics can there be respectively attributed by the other methodologies.

Thus, in ir, the carbonyl vibration peaks were setting between 1760-1750 cm^{-1} for the **3** type compounds, as described [5], then for the **4** type compounds they appeared above 1660 cm^{-1}. In ms, only opened products, **4**, gave a dehydration peak (M-18) whereas the other fragmentations were nearly similar. ^{1}H-nmr irradiation experiments demonstrated that 5-H signal is less shielded than 8-H signal for compounds **3a** [1] and **3e** as opposed with that was obtained for compounds without heterocycle **4b** [2], **4c** [2] and **4d**; so that for the two equivalent hydrogens 6-H and 3-H, the signal of the latter is then less shielded. The hydrogen in 6-position is likely more apart of the shielding zone of the 1-carbonyl group which engages a hydrogen bond, **5**.

The fungitoxicity of Dianthalexin on the filamentous fungus *Cladosporium herbarum* was established with the Keen's tlc test [6]. Similar assays have showed no fungitoxicity for $\mathbf{3a_0}$, $\mathbf{3f_0}$ and $\mathbf{3h_0}$ while their three hydroxylated homologues **4a, 4f** and **4h** were enough fungitoxic.

Confirmation and valuation of these qualitative tests should supply information to establish a structure-activity relation for this new group of active substances.

EXPERIMENTAL

Melting points were determined using a Leitz hot stage microscope and are uncorrected. The uv spectra were recorded in methanol on a Jobin et Yvon 201 instrument, λ max and λ min are given, sh = shoulder. The ir spectra were recorded from potassium bromide on a Perkin-Elmer Infracord instrument. The ms spectra were taken on a MS 902 spectrometer at 70 eV ionizing beam. The ^{1}H-nmr were recorded on a Cameca (360 MHz) instrument with tetramethylsilane as an internal standard, J_0 = *ortho* coupling, Jm = *meta* coupling.

Synthesis of 4*H*-3,1-Benzoxazin-4-ones $\mathbf{3a_0}$-$\mathbf{h_0}$ and **3e**. General Procedure.

A mixture of one anthranilic acids **1a-d** (1 mmole) and aroyl chloride **2a** or **2b** (2.5-3.5 mmoles) in dry pyridine (20 ml) was stirred during 4-6 hours. The mixture was then cooled. The precipitate, isolated by suction, was washed with water and recrystallized.

2-Phenyl-7-benzoyloxy-4*H*-3,1-benzoxazin-4-one ($\mathbf{3a_0}$).

A mixture of **1b** (0.5 g, 3.2 mmoles) and **2a** (1.6 g, 11.4 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from acetone, gave $\mathbf{3a_0}$ (1 g, 89%) mp 166-167°; ir: ν 1750, 1730 (>C=O) cm^{-1}; uv: λ (ε) 221 (19379), 243 (31178), 270 sh (15435), 284 sh (9261), 294 (6105), 309 (7374), 340 (342) nm.

Anal. Calcd. for $C_{21}H_{13}NO_4$: C, 73.46; H, 3.82; N, 4.08. Found: C, 73.74; H, 3.84; N, 3.88.

2-(2-Acetyloxyphenyl)-7-methoxy-4*H*-3,1-benzoxazin-4-one ($\mathbf{3b_0}$).

A mixture of **1c** (0.3 g, 1.8 mmoles) and **2b** (1 g, 5.0 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from ethyl acetate, gave $\mathbf{3b_0}$ (0.43 g, 78%) mp 166-167°; ir: ν 1745, 1730 (>C=O) cm^{-1}; uv: λ (ε) 230 (23636), 257 (92678), 279 (22392), 294 (25190) nm; ms: m/z (%) 311 (M^+, 4), 269 (100),

255 (12), 176 (8), 149 (48), 121 (12), 120 (14), 92 (7), 77 (5).

Anal. Calcd. for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.21; N, 4.50. Found: C, 65.72; H, 4.30; N, 4.22.

2-(2-Acetyloxyphenyl)-7-(2-acetyloxybenzoyloxy)-4*H*-3,1-benzoxazin-4-one (**3d$_0$**).

A mixture of **1b** (0.9 g, 5.8 mmoles) and **2b** (4.4 g, 22.1 mmoles) was treated according to the general procedure. The obtained precipitate, recrystallized from ethyl acetate and acetic acid, gave **3d$_0$** (1.8 g, 68%) mp 182-183°; ir: ν 1755, 1740 (>C=O) cm^{-1}; uv: λ (ϵ) 227 (32588), 246 (53244), 271 (22950), 284 (24786), 298 sh (20378), 318 sh (11934), 340 (459) nm; ms: m/z (%) 417 (M-42,6), 163 (75), 121 (100).

Anal. Calcd. for $C_{25}H_{17}NO_8$: C, 65.36; H, 3.73; N, 3.05. Found: C, 65.43; H, 3.88; N, 2.87.

2-Phenyl-8-benzoyloxy-4*H*-3,1-benzoxazin-4-one (**3f$_0$**).

A mixture of **1d** (1.5 g, 9.8 mmoles) and **2a** (4.5 g, 32.0 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from ethyl acetate, gave **3f$_0$** (2 g, 74%) mp 176-177°; ir: ν 1755, 1720 (>C=O) cm^{-1}; uv: λ (ϵ) 232 (31899), 271 (7134), 274 (7168), 279 (6688), 285 (6997), 296 (5693), 299 (5831), 327 sh (2744), 342 sh (1440), 350 (343) nm; ms: m/z (%) 343 (M^+, 5), 149 (5), 105 (100), 77 (28).

Anal. Calcd. for $C_{21}H_{13}NO_4$: C, 73.46; H, 3.82; N, 4.08. Found: C, 73.57; H, 3.93; N, 3.83.

2-(2-Acetyloxyphenyl)-8-(2-acetyloxybenzoyloxy)-4*H*-3,1-benzoxazin-4-one (**3g$_0$**).

A mixture of **1d** (2 g, 13.0 mmoles) and **2b** (8 g, 40.3 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from acetone, gave **3g$_0$** (1.45 g, 24%) mp 159-160°; ir: ν 1775, 1750, 1735, 1695 (>C=O) cm^{-1}; uv: λ (ϵ) 221 (48011), 239 (63525), 259 (38556), 279 (45900), 320 sh (17533), 360 (1377) nm; ms: m/z (%) 417 (M-42,3), 360 (7), 297 (10), 255 (19), 240 (28), 237 (11), 163 (60), 121 (100), 92 (22).

Anal. Calcd. for $C_{25}H_{17}NO_8$: C, 65.36; H, 3.73; N, 3.05. Found: C, 65.66; H, 3.83; N, 2.75.

2-(2-Acetyloxyphenyl)-4*H*-3,1-benzoxazin-4-one (**3h$_0$**).

A mixture of **1a** (2.5 g, 18.2 mmoles) and **2b** (5 g, 54.6 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from acetone-water (2:1), gave **3h$_0$** (2.8 g, 55%) mp 149-150°; ir: ν 1750 (>C=O) cm^{-1}; uv: λ (ϵ) 224 (20007), 230 (20794), 250 (10116), 263 (12026), 288 (6125), 311 (9778), 350 (505) nm; ms: m/z (%) 281 (M^+, 3), 239 (100), 121 (17), 119 (35), 92 (10).

Anal. Calcd. for $C_{16}H_{11}NO_4$: C, 68.32; H, 3.94; N, 4.98. Found: C, 68.50; H, 4.09; N, 4.71.

2-Phenyl-7-hydroxy-4*H*-3,1-benzoxazin-4-one (**3a**, Dianthalexin).

The compound **3a$_0$** (0.97 g, 2.8 mmoles) dissolved in dimethylformamide (20 ml) was stirred with potassium *t*-butoxide (0.49 g, 4.4 mmoles) during 1 hour. The mixture was then diluted and neutralized with acetic acid. The precipitate isolated by suction, washed with water and twice recrystallized from dichloromethane-acetone (3:1) gave **3a** (0.2 g, 29%) mp 231-232° (lit [1] 229-231°).

2-Phenyl-7-methoxy-4*H*-3,1-benzoxazin-4-one (**3e**).

A mixture of **1c** (0.3 g, 1.8 mmoles) and **2a** (0.6 g, 4.2 mmoles) was treated according to the general procedure. The obtained precipitate, twice recrystallized from ethyl acetate, gave **3e** (0.25 g, 55%) mp 152-153 (lit [7] 150.7-151.3°); ms: m/z (%) 253 (M^+, 100), 210 (13), 209 (61), 176 (45), 120 (15), 106 (29), 105 (40), 77 (56); ^{1}H-nmr (deuteriochloroform): δ 3.93 (s, 3H, 7-OCH_3), 7.05 (dd, 1H, J_0 = 8 Hz, Jm = 2 Hz, 6-H), 7.08 (d, 1H, Jm = 2 Hz, 8-H), 7.40-7.65 (m, 3H, 3', 4', 5'-H), 8.40-8.80 (m, 3H, 2', 5,6'-H) ppm.

2-Phenyl-8-hydroxy-4*H*-3,1-benzoxazin-4-one (**3f**).

The compound **4f** (30 mg) was melted at 140-150° during 1 hour. The obtained product, recrystallized from ethyl acetate, gave **3f** (25 mg, 89%) mp 161-162°; ir: ν 1670 (>C=O) cm^{-1}; uv: λ (ϵ) 228 (11113), 241 (14053), 256 (12021), 265 (13264), 275 (13862), 280 (13623), 307 (21510), 340 (478) nm; ms: m/z (%) 239 (M^+, 44), 195 (100), 105 (16), 77 (16).

Anal. Calcd. for $C_{14}H_9NO_3$: C, 70.29; H, 3.79; N, 5.86. Found: C, 70.37; H, 3.82; N, 5.64.

2-(2-Hydroxyphenyl)-8-hydroxy-4*H*-3,1-benzoxazin-4-one (**3g**).

The compound **4g** (28 mg) was melted at 160-170°, *in vacuo* during 2 hours. The obtained product recrystallized from ethyl acetate, gave **3g** (13 mg, 50%) mp 237-238°; uv: λ (ϵ) 230 (13515), 242 (16600), 249 (14790), 255 (16126), 257 (16039), 262 (16626), 270 sh (14790), 285 (9562), 292 sh (11347), 303 (15427), 309 (14280), 330 (23842), 340 sh (21930), 370 (1147), 386 sh (892), 420 (255) nm; ms: m/z (%) 255 (M^+, 46), 237 (100), 209 (27), 181 (11), 153 (6), 121 (7), 107 (17), 105 (6), 77 (11).

Anal. Calcd. for $C_{14}H_9NO_4$: C, 65.88; H, 3.55; N, 5.49. Found: C, 65.98; H, 3.69; N, 5.32.

Synthesis of 2-Aroylamino-aromatic Acids **4b-h**. General Procedure.

The compounds **3b$_0$-h$_0$** (1 mmole) dissolved in acetone (20 ml) and 2*N* sodium hydroxide (3-4 mmoles) were stirred until the starting material can no longer be detected by tlc. The mixture was then neutralized with acetic acid. Acetone was removed *in vacuo* and the formed precipitate was isolated by suction and recrystallized.

2-(2-Hydroxybenzoyl)amino-4-methoxybenzoic Acid (**4b**, Dianthramide A).

The compound **3b$_0$** (0.4 g) was treated according to the general procedure. The obtained precipitate, twice recrystallized from acetone, gave **4b** (0.25 g, 68%) mp 216-217° [2].

2-(2-Hydroxybenzoyl)amino-4-hydroxybenzoic Acid (**4d**).

The compound **3d$_0$** (0.65 g) was treated according to the general procedure. The obtained precipitate, twice recrystallized from ethanol, gave **4d** (0.25 g, 71%) mp 262-264°; ir: ν 1650, 1630 (>C=O) cm^{-1}; uv: λ (ϵ) 232 (15288), 253 (33961), 278 (13158), 285 (13377), 294 (13104), 318 (16188), 350 (955) nm; ms: m/z (%) 273 (M^+, 20), 255 (54), 229 (9), 153 (44), 135 (48), 121 (100), 109 (48); ^{1}H-nmr (deuterioacetone): δ = 6.73 (dd, 1H, J_0 = 8 Hz, Jm = 2 Hz, 5-H), 7.02 (m, 2H, 3',5'-H), 7.52 (t, 1H, J_0 = 8 Hz, 4'-H), 7.85 (d, 1H, J_0 = 8 Hz, 6'-H), 8.08 (d, 1H, J_0 = 8 Hz, 6-H), 8.39 (d, 1H, Jm = 2,3-H), 12.20 (s, 1H, -COOH), 12.78 (s, 1H, 2'-OH) ppm.

Anal. Calcd. for $C_{14}H_{11}NO_5$: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.70; H, 4.17; N, 5.04.

2-Benzoylamino-3-hydroxybenzoic Acid (**4f**).

The compound **3f$_0$** (0.6 g) was treated according to the general procedure. The obtained precipitate, twice recrystallized from benzene, gave **4f** (0.27 g, 64%) mp 169-170°; ir: ν 1655, 1625 (>C=O) cm^{-1}; uv: λ (ϵ) 230 (18632), 250 (9766), 270 sh (7710), 293 (5088), 319 (7401), 370 (514 nm; ^{1}H-nmr (deuterioacetone): δ 7.25 (m, 2H, 4,5-H), 7.7 (m, 3H, 3',4',5'-H), 8.01 (m, 3H, 2', 6,6'-H), 12.25 (s, 1H, -COOH) ppm; ms: m/z (%) 257 (M^+, 7), 239 (5), 195 (7), 105 (100), 77 (31).

Anal. Calcd. for $C_{14}H_{11}NO_4$: C, 65.36; H, 4.31; N, 5.45. Found: C, 65.60; H, 4.48; N, 5.31.

2-(2-Hydroxybenzoyl)amino-3-hydroxybenzoic Acid (**4g**).

The compound **3g$_0$** (1 g) was treated according to the general procedure. The obtained precipitate, twice recrystallized from ethyl acetate, gave **4g** (0.3 g, 47%) mp 200-202°; ir: ν 1660 (>C=O) cm^{-1}; uv: λ (ϵ) 237 (22659), 260 sh (12558), 282 (7917), 317 (13650), 380 (136) nm; ms: mz (%) 273 (M^+, 13), 153 (21), 135 (12), 121 (100), 93 (12).

Anal. Calcd. for $C_{14}H_{11}NO_5$: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.62; H, 4.28; N, 4.97.

2-(2-Hydroxybenzoyl)aminobenzoic Acid (**4h**).

The compound **3h$_0$** (2.2 g) was treated according to the general procedure. The obtained precipitate, twice recrystallized from acetone, gave

4h (0.8 g, 40%) mp 219-220° (lit [8] 217-218°); ms: mz (%) 257 (M^+, 37), 239 (16), 137 (100), 121 (73), 120 (27), 119 (54), 92 (17).

REFERENCES AND NOTES

[1] M. L. Bouillant, J. Favre-Bonvin and P. Ricci, *Tetrahedron Letters*, **24**, 51 (1983).

[2] M. Ponchet, J. Martin-Tanguy, A. Marais and A. Poupet, *Phytochem.*, **23**, 1901 (1984).

[3] S. Mayama, T. Tani, T. Ueno, K. Hirabayashi, T. Nakashima, M. Fukami, Y. Mizuno and H. Irie, *Tetrahedron Letters*, **22**, 2103 (1981).

[4] S. Mayama, T. Tani, Y. Matsuura, T. Ueno, R. Hirabayashi, H. Fukami, Y. Mizuno and H. Irie, *J. Chem. Soc. Japan*, **5**, 697 (1981).

[5] D. I. Bain and R. K. Smalley, *J. Chem. Soc.* (C), 1593 (1968).

[6] N. T. Keen, *Physiol. Plant Pathol.*, **1**, 265 (1971).

[7] M. V. Loseva, B. M. Bolotin and R. N. Nurmukhametov, *Russ. J. of Phys. Chem.*, **45**, 176 (1971).

[8] *Chem. Abstr.*, **70**, p 87330z (1969).