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NEW NAPHTHOPYRONES FROM ASPERGILLUS FONSECAEUS

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Abstract - Four new dimeric naphthopyrones, fonsecinones A (7), B (8), C (9), and D (10), and two known ones, aurosperones A (6) and B (11), were isolated from Aspergillus fonsecaeus (N.R.R.L. 67, O 16-1).

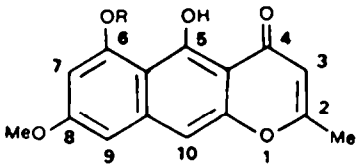
Naphtho- -pyrones have been isolated from a variety of fungi, e.g. rubrofusarin (1) from Fusarium culmorum,[1-3] Fusarium graminеum[1-3] and Aspergillus niger,[4] flavasperone (3) from A. niger,[4-6] Aspergillus awamori[6] and Aspergillus fonsecaeus (N.R.R.L., N° 67),[5] and the dimeric aurosperones A (6) and B (11) from A. niger and A. awamori.[4,6,7] The presence of naphthopyrones in the extractives of fruits infected by A. niger was also established by Ghosal et al. Because of their toxicity, such materials may provide high toxin risk in man.[4]

Raper and Fennell[9] described an ultraviolet mutant (O 16-1) of Aspergillus fonsecaeus (N.R.R.L. 67), a fungus closely related to A. niger, which was characterized by a rich deposit of yellow pigment. A previous investigation established the occurrence of fonsecin (4), fonsecin B (5) and rubrofusarin B (2) in this material.[10,11] This report deals with the isolation and characterization of six further pigments from the same source. Extensive TLC over Si gel of the EtOAc extracts of dried mycelium of Aspergillus fonsecaeus (N.R.R.L. 67, O 16-1) afforded the new dimeric naphthopyrones, named as fonsecinones A (7), B (8), C (9) and D (10), in addition to two known ones, viz. aurosperones A (6) and B (11). Their structure was evident from inspection of their spectroscopic parameters, particularly PMR characteristics (Table 2). Comparison with standard spectra (Table 1) and use of a pair of solvents facilitated proton shift assignments.

The identity of compounds 6 $C_{32}H_{26}O_{10}$ (M^+; 570), and 11, $C_{32}H_{30}O_{12}.H_2O$ ($M^+ - H_2O$; 588), with aurosperones A and B, respectively, was established by correspondence of their spectral data with those reported by Tanaka et al.[6,7] The MS, UV and PMR spectral data of fonsecinones B (8) and D (10), both $C_{32}H_{28}O_{11}\cdot 0.5\ H_2O$ ($M^+ - H_2O$; 570), indicated them to be unsymmetric dimers involving rubrofusarin B (2) and fonsecin B (5) as monmeric halves and formation of aurosperone A (6) by dehydration confirmed a C(7)-C(10') linking pattern between the two moieties. In the PMR spectrum of fonsecinone B (8), the signals

for 2-Me and 5-OH, as compared with those of aurosperone B (11), are low-field shifted to 2.44 and 14.84 ppm (DMSO-d_6 solutions), i.e. positions at which resonate 2-Me and 5-OH in aurosperone A (6). In contrast, 2'-Me and 5'-OH of fonsecinone D (10) move downfield to 2.17 and 15.09 ppm, which are close to 2.13 and 15.11 ppm of 2'-Me and 5'-OH resonances of aurosperone A (6). Thus, fonsecinones B and D can be formulated as 2- and 2'-anhydroaurosperone B, respectively. Ghosal et at.[4] have isolated a dimeric naphthopyrone from A. niger, named as aurosperone E, for which structure 10 was proposed. A clear sorrelation between its spectral data and those of fonsecinone D could not be found.

Fonsecinone A (7), $C_{32}H_{26}O_{10}$ (M^+; 570), is structurally related to fonsecinone C (9), $C_{32}H_{28}O_{11} \cdot 0.5\ H_2O$ (M^+; 588), from which can be derived by dehydration. Both possess an angular naphthopyrone moiety as indicated by the high field position of one of the chelated hydrogens in the PMR spectrum and marked spectral changes in the UV pattern. UV differences between linear and angular series of naphthopyrones derivatives have been well established by Bycroft et al.[5] and Fukushima et al.[12] The main absorptions of linear naphthopyrones are located at ca 225, 280, 325 and 400 nm, while angular compounds such as flavasperone (3) absorb at 241, 282 and 370 nm. The UV spectra of 7 and 9 combines the features of simple rubrofusarin B and flavasperone chromophores. There is no absorption maximum at 325 nm, but shoulders on the slope of the 280 nm band, in addition to an increase in molecular extinction at 240 nm and an hypsochromic shift of the 400 nm band. Results which are in accordance with the presence of an angular moiety. Consistently, PMR data show that the 7-linked rubrofusarin B moiety in aurosperone A (6) and fonsecinone B (8) has isomerized to the flavasperone structure in fonsecinones A (7) and C (9) as indicated by the downfield shift of 2-Me and 3-H and the highfield shift of 10-H and 5-OH. This findings are compatible with structures 7 and 9 for fonsecinones A and C, respectively.



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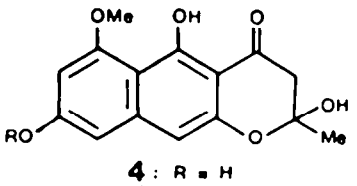

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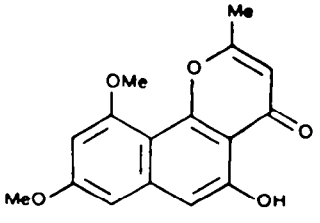

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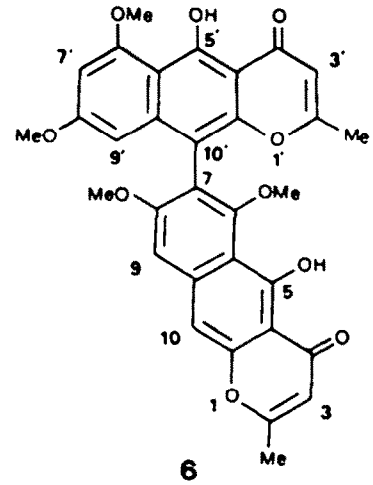
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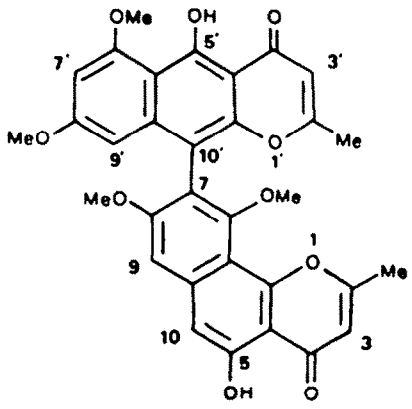
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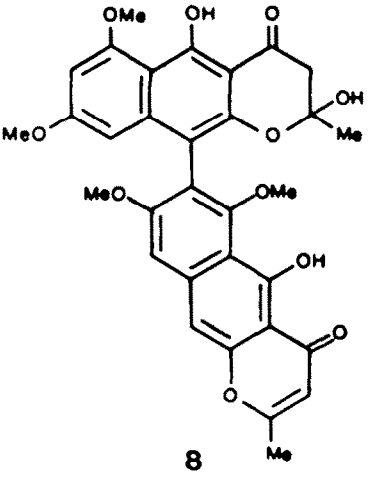
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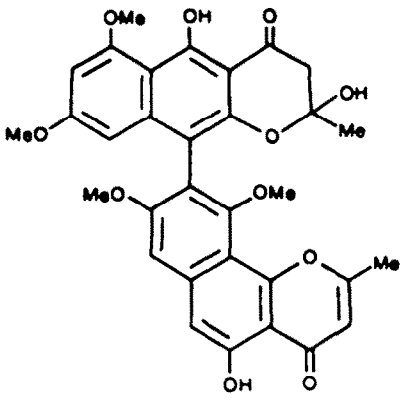
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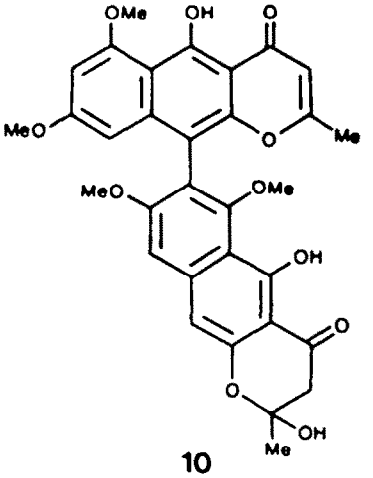
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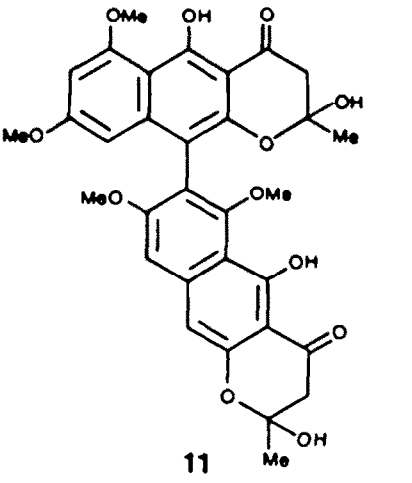
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11

Examination of PMR data shows that the signals for the Me, aromatic and phenolic protons of the rubrofusarin B, flavasperone and fonsecin B moieties in dimers are rather unaffected by modifications in the other half molecule. These protons exhibit a narrow resonance range and characteristic positions in the spectra (Table 3). Because of diamagnetic fields induced in the naphthalenoid ring systems, all protons located above or below the plane of the other half of the molecule are abnormally shielded. As compared with resonances of the corresponding protons in monomers, 2'-Me, 9'-H, 6-OMe, 8-Me and 8'-OMe are significantly shifted to higher fields. Alternatively, a paramagnetic shift of 9-H and 10-H is observed. Because of strong intramolecular hydrogen bonding to the carbonyl group, the phenolic protons resonate at low fields. The chelate ring is associated with the 1,2-bond of the naphthalene structure in linear naphthopyrones while with the 2,3-bond in the angular ones. The decreased deshielding experienced by the protons of the OH group in the flavasperone moiety of fonsecinones A and C as compared with those in linear structures may be explained in terms of interaction between these two ring systems. The 1,2-bond of naphthalene has more double bond character than the 2,3-bond.[13,14] Since intramolecular hydrogen bonding effects occur in six-membered rings associated with two double bonds,[15] a strongest hydrogen bonding and deshielding of phenolic protons results in linear structures. Differential deshielding of chelated protons was also observed in linear and angular furanoflavones.[16]

Table 1. ^{1}H Chemical shifts of rubrofurarin B (2), fonsecin (4) and fonsecin B (5).

	2		4		5	
	$CDCl_3$	DMSO-d_6	DMSO-d_6	$(CD_3)_2CO$	DMSO-d_6	$(CD_3)_2CO$
2-Me	2.37	2.36	1.67	1.72	1.62	1.66
3-H	5.94	6.12	-	-	-	-
3-H_2	-	-	2.74[b]	2.85[d]	2.83[e]	g
			3.14[b]	3.07[d]	3.07[e]	
7-H	6.35[a]	6.43[a]	6.33[a]	6.42[a]	6.37[a]	6.37[a]
9-H	6.51[a]	6.78[a]	6.50[a]	6.60[a]	6.69[a]	6.65[a]
10-H	6.87	7.09	6.48	6.44	6.56	6.53
6-OMe	4.02	3.88	3.90	3.87	3.85	3.89
8-OMe	3.92	3.88	-	-	3.85	3.87
5-OH	14.86	14.77	14.21[c]	n.r.	14.14[f]	n.r.

Spectra taken at 60 MHz, except for 4 in DMSO-d_6 taken at 80 MHz; δ, ppm from TMS. [a] $J_{7,9}$ = 2.5 Hz. [b] J_{gem} = 17.2 Hz. [c] 2-OH and 8-OH at 6.97 and 10.20 ppm. [d] J_{gem} = 17.7 Hz. [e] Position of inner lines. [f] 2-OH at 7.01 ppm. [g] Signals obscured because of overlapping with H_2O peak. n.r. = not recorded.

Table 2. ^{1}H Chemical shifts of compounds 6-11.

	6		7		8		9		10		11	
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6
2-Me	2.38	2.42	2.48	2.49	2.39	2.44	2.43	2.52	1.78	1.72	1.75	1.69
3-H	5.97	6.19	6.33	6.52	6.01	6.19	6.30	6.48	-	-	-	-
3-H_2	-	-	-	-	-	-	-	-	3.01	2.97	2.99	2.96
9-H	6.97	7.33	6.98	7.33	6.95	7.22	7.01	7.21	6.84	7.09	6.83	7.12
10-H	7.15	7.36	7.06	7.14	7.10	7.30	7.01	7.05	6.70	6.79	6.69	6.79
6-OMe	3.46	3.40	3.44	3.42	3.43	3.37	3.43	3.35	3.42	3.40	3.42	3.39
8-OMe	3.77	3.77	3.78	3.78	3.80	3.77	3.82	3.76	3.74	3.77	3.76	3.76
5-OH	14.97	15.00	12.81	12.97	14.70	14.84	12.76	12.85	14.11	14.15	14.23	14.25
2'-Me	2.10	2.13	2.12	2.13	1.49	1.40	1.45	1.42	2.11	2.17	1.45	1.44
3'-H	6.03	6.22	6.01	6.21	-	-	-	-	5.98	6.18	-	-
3'-H_2	-	-	-	-	2.91	2.88 3.00	2.93	2.90 3.01	-	-	2.89	2.96
7'-H	6.42	6.56	6.44	6.57	6.35	6.36	6.41	6.39	6.42	6.54	6.35	6.43
9'-H	6.22	6.17	6.22	6.17	6.13	5.92	6.18	5.92	6.24	6.18	6.14	6.02
6'-OMe	4.00	3.94	4.02	3.95	3.98	3.92	3.99	3.90	4.00	3.95	3.95	3.93
8'-OMe	3.61	3.59	3.62	3.60	3.62	3.56	3.63	3.54	3.63	3.63	3.62	3.59
5'-OH	15.21	15.11	15.22	15.14	14.40	14.28	14.63	14.33	15.10	15.09	14.51	14.44

Spectra taken at 60 MHz; δ, ppm from TMS; $J_{7',9'}$ = 2.5 Hz. Here, as well as text and Table 3, the flavasperone systems of compounds 7 and 9 are numbered as for 2 and 5 for the sake of consistency; the correct numbering is given in the Experimental.

Table 3. Resonance range for protons of the rubrofusarin B (2), flavasperone (3) and fonsecin B (5) moieties in compounds 6-11.

	2-CH_3	2'-CH_3	3-H	3'-H	7'-H	9-H	9'-H	10-H	5-OH	5'-OH
					$CDCl_3$ solution					
2	2.38-2.39	2.10-2.12	5.97-6.01	5.98-6.03	6.42-6.44	6.95-6.97	6.22-6.24	7.10-7.15	14.70-14.97	15.10-15.22
3	2.43-2.48	-	6.30-6.33	-	-	6.98-7.01	-	7.01-7.06	12.76-12.81	-
5	1.75-1.78	1.45-1.49	-	-	6.35-6.41	6.83-6.84	6.13-6.18	6.69-6.70	14.11-14.23	14.40-14.63
					DMSO-d_6 solution					
2	2.42-2.44	2.13-2.17	6.19	6.18-6.22	6.54-6.57	7.22-7.33	6.17-6.18	7.30-7.36	14.84-15.00	15.09-15.14
3	2.49-2.52	-	6.48-6.52	-	-	7.21-7.33	-	7.05-7.14	12.85-12.97	-
5	1.69-1.72	1.40-1.44	-	-	6.36-6.43	7.09-7.12	5.92-6.02	6.79	14.15-14.25	14.28-14.44

In the 60 MHz spectrum of fonsecin in H_2O-free $(CD_3)_2CO$ solution and also in the 80 MHz spectrum in DMSO-d_6 solution, the isolated CH_2 group of the dihydro- -pyrone ring is recognized at ca. 3 ppm as a characteristic four line AB pattern. In the 60 MHz spectrum of fonsecin, as well as fonsecin B and the fonsecinones B and C, only the intense inner satellites of the AB system are observed, the weak outer satellites being hidden under the H_2O and DMSO-d_5 absorptions. In the spectra of fonsecinone D and aurosperone B, the geminal resonances were unresolved. The non-equivalence of the geminal protons is lost in $CDCl_3$ solutions.

The shift assignments for OMe resonances were based on those reported for aurosperones by Tanaka et al.[8] from benzene-induced solvent shift studies. It can be seen from Tables 1-3 that pronounced proton shifts also occur on change of solvent from $CDCl_3$ to DMSO-d_6. Of interest is the behaviour of the aromatic protons of dimers 6-11. The signals for 3-H, 3'-H, 7'-H, 10-H and particularly 9-H move downfield, whereas 9'-H, flanked by a naphthopyrone unit, is rather shielded in DMSO-d_6 solutions.

In the MS, hydrated fonsecinones easily loss elements of H_2O showing intense M - H_2O peaks at m/e 570 which frequently represent the bse peak. Conspicuous peaks at m/e 539 and 540 result from loss of $CH_3O^{\cdot}$ and CH_2O from the OMe groups, while the m/e 503 and 504 fragments are probably formed by cleavage of a -pyrone ring with expulsion of CH_3-C=C=C=O and CH_2=C=C=C=O. A characteristic peak at m/e 285 is found in the spectra of all naphthopyrone dimers. These fragments correspond to half molecules because of rupture of the C(7)-C(10') bond linking them; such fragmentation has been noted previously.[4]

Aurosperone B was earlier found to dehydrate by HCl-treatment to give aurosperone A.[6] In the present work, the methods of Traynelis et al.[17] (DMSO soln, 170°, 8 h) and Bible and Atwater[18] (MeCOEt soln, Al_2O_3, 80°, 2 h) for the dehydration of alcohols were employed. Under these conditions, fonsecinone B, fonsecinone D and aurosperone B afford aurosperone A, while fonsecinone C gives fonsecinone A; dehydrations which parallel that of fonsecin B to rubrofusarin B.[11] The fact that aurosperone B is the principal dimeric pigment of A. fonsecaeus (N.R.R.L. 67, O 16-1) and is easily dehydrated suggests that the fonsecinones may be an artefact. However, they are detected in the pigments-extracts at mild conditions when monitored by TLC and, as well as fonsecin, fonsecin B and aurosperone B, were stable when subjected to preparative chromatography. That aurosperone A and the fonsecinones are natural products is further supported by Tanaka et al.[6] who found differences in the pigments contents-ratio among different strains of A. niger and A. awamori.

The present investigation has revealed that the pigment deposit of A. fonsecaeus (N.R.R.L. 67, O 16-1) is rich in dimeric naphthopyrones of various types. It is interesting to note that while flavasperone is the principal pigment of A. fonsecaeus (N.R.R.L. 67),[5] its ultraviolet mutant (O 16-1) accumulates mainly fonsecin and aurosperone B. Flavasperone itself could not be isolated from the latter, but its structure is present in fonsecinones A and C. The new dimeric metabolites of A. fonsecaeus (N.R.R.L. 67, O 16-1) described in this paper also belong to the poliketide group and can be formulated as biosynthetically derived from monomeric intermediates by phenol oxidative coupling. From the taxonomical point of view, this strain is closely related to A. niger and A. awamori, active producers of aurosperone B.[6]

Naphthopyrones are mycotoxins which may be present in food stuffs infected

with Aspergillus species related to A. niger. Their toxicity was first evaluated by Ghosal et al.[4] who found that the total naphthopyrones isolated from the mycelial extracts of A. niger van. Tiegh and from mango fruits naturally infected with the same strain produce marked central nervous system depressant effect in albino mice and rats leading to death by respiratory failure. Ingestion of contaminated food such as mango fruits and derived materials may cause mental deficiencies or predispose man to other ailments.[4] It has been suggested that naphthopyrones have a close relation to the formation of black humic substance in A. niger and A. awamori. Their role in the metabolism of these aspergilli is uncertain, though inhibitory activity on mitochondrial oxidative phosphorylation and on a hydrolase has been claimed.[8]

EXPERIMENTAL

Mp's were determined on a Kofler block and are uncorrected. UV spectra were carried out in 95% EtOH and IR spectra in KBr discs. PMR spectra were taken at 60 MHz, unless otherwise stated, using TMS as internal standard. MS spectra were obtained in a Varian Mat CH7 spectrometer at 70 eV. Analytical and preparative TLC was performed on Si gel in (1) $CHCl_3$-MeOH (98:2), two developments (compound, R_f: 2, 0.85; 6, 0.76; 7, 0.76; 5, 0.71; 8, 0.66; 9, 0.60; 10, 0.53; 11, 0.32; 4, 0.20), and (2) C_6H_6-MeCOEt (90:10), two developments (compound, R_f: 5, 0.65; 6, 0.61; 7, 0.47; 8, 0.36; 9, 0.28).

Extraction and Isolations. The mycelium of A. fonsecaeus (N.R.R.L. 67, O 16-1) was extraced with EtOAc as previously reported.[10] Evaporation of the EtOAc extracts gave a brown solid (4.18 g) which was subjected to repeated prep. TLC (systems 1 and 2). The bands were eluted with $CHCl_3$-MeOH (90:10) to give compounds 6-11.

5,5'-Dihydroxy-6,6',8,8'-tetramethoxy-2,2'-dimethyl-(7,10'-Bi-4H-naphtho(2,3-b)pyran)-4,4'-dione, aurosperone A (6). Yellow micro-plates ($CHCl_3$/n-PrOH), 84 mg, m.p. 290-291°; UV λ_{max} nm (log ε): 223 (4.62), 255 sh (4.66), 278 (4.93), 325 (3.98), 401 (4.01); IR γ_{max} cm^{-1}: 1650, 1615, 1585, 1408, 1248, 1160, 1098; EIMS, m/z (rel. int.): 570 (M^+) (100), 539 (6), 538 (5), 513 (7), 286 (13), 285 (9), 270 (8), 269 (18), 268 (7), 257 (5). (Found: C, 67.46; H, 4.62. Calcd for $C_{32}H_{26}O_{10}$: C, 67.37; H, 4.56%).

5-Hydroxy-9-(5-hydroxy-6,8-dimethoxy-2-methyl-4-oxo-4H-naphtho(2,3-b)pyran-10-yl)-8,10-dimethoxy-2-methyl-4Hnaphtho(1,2-b)pyran-4-one, fonsecinone A (7). Yellow micro-needles ($CHCl_3$/n-PrOH), 86 mg, m.p. 280°; UV λ_{max} nm (log ε): 228 (4.66), 256 (4.65), 278 (4.83), 325 sh (4.06), 398 (3.85); IR γ_{max} cm^{-1}: 1650, 1608, 1584, 1420, 1408, 1280, 1262, 1230, 1209, 1160; EIMS m/z (rel. int.): 570 (M^+) (100), 539 (2), 513 (5), 512 (4), 285 (8), 269 (2). (Found: C, 67.00; H, 4.72. Calcd. for $C_{32}H_{26}O_{10}$: C, 67.37; H, 4.56%).

2',3'-Dihydro-2',5,5'-trihydroxy-6,6',8,8'-tetramethoxy-2,2'-dimethyl-(7,10'-Bi-4H-naphtho(2,3-b)pyran)-4,4'-dione, fonsecinone B (8). Amorphous solid obtained by addition of H_2O to a MeOH solution, 28 mg, m.p. 172-173°; UV λ_{max} nm (log ε): 229 (4.59), 255 sh (4.62), 280 (4.85), 320 (4.15), 328 sh (4.15), 403 (3.96); IR γ_{max} cm^{-1}: 1615, 1580, 1418, 1235, 1197, 1158, 1092, 1076, 1008; EIMS, m/z (rel. int.): 570 $(M - H_2O)^+$ (95), 541 (10), 540 (32), 539 (100), 538 (14), 524 (11), 472 (12), 299 (12), 286 (15), 285 (8), 270 (19), 269 (13). (Found: C, 63.89; H, 5.08. Calcd for $C_{32}H_{28}O_{11} \cdot 0.5\ H_2O$: C, 64.32; H, 4.86%).

5-Hydroxy-9-(2,3-dihydro-2,5-dihydroxy-6,8-dimethoxy-2-methyl-4-oxo-4H-naphtho-(2,3-b)pyran-10-yl)-8,10-dimethoxy-2-methyl-4H-naphtho(1,2-b)pyran-4-one, fonsecinone C (9). Amorphous precipitate obtained by addition of H_2O to a MeOH solution, 39 mg, m.p. 169-170°; UV λ_{max} nm (log ε): 234 (4.71), 254 (4.58), 279 (4.78), 315 sh (4.29), 327 sh (4.17), 398 (3.85); IR γ_{max} cm^{-1}: 1660, 1605, 1575, 1404, 1378, 1307, 1228, 1155, 1112; EIMS, m/z (rel. int.): 588 $(M)^+$ (22), 572 (8), 571 (34), 570 (100), 556 (5), 541 (7), 540 (16), 539 (39), 513 (5), 505 (11), 504 (32), 499 (10), 299 (13), 285 (9), 270 (14). (Found: C, 64.15; H, 5.19. Calcd for $C_{32}H_{28}O_{11} \cdot 0.5\ H_2O$: C, 64.32; H, 4.86%).

2,3-Dihydro-2,5,5'-trihydroxy-6,6',8,8'-tetramethoxy-2,2'-dimethyl-(7,10'-Bi-4H-naphtho(2,3-b)pyran)-4,4'-dione, fonsecinone D (10). Solid residue, 50 mg, m.p. 166-170°; UV λ_{max} nm (log ε): 227 (4.43), 279 (4.69), 316 sh (3.91), 328 sh (3.84), 403 (3.80); IR γ_{max} cm^{-1}: 1605, 1580, 1398, 1220, 1152, 1090, 1050, 1005; EIMS, m/z (rel. int.): 570 $(M - H_2O)^+$ (100), 557 (6), 539 (7), 513 (7), 504 (9), 286 (8), 285 (8), 269 (13).

2,2',3,3'-Tetrahydro-2,2',5,5'-tetrahydroxy-6,6',8,8'-tetramethoxy-2,2'-dimethyl-(7,10'-Bi-4H-naphtho(2,3-b)pyran)-4,4'-dione, aurosperone B (11). Amorphous solid obtained by addition of H_2O to a MeOH solution, 420 mg, m.p. 175-176°; UV λ_{max} nm (log ε): 233 (4.63), 270 sh (4.73), 280 (4.84), 318 (4.22), 331 (4.22), 404 (4.01); IR γ_{max} cm^{-1}: 1615, 1410, 1243, 1170, 1123,

1100, 1062, 1022; EIMS, m/z (rel. int.): 588 $(M - H_2O)^+$ (5), 570 (100), 542 (8), 541 (20), 540 (58), 504 (9), 503 (29), 474 (13), 300 (17), 286 (8), 285 (12), 270 (22), 269 (8), 236 (9),. (Found: C, 61.48; H, 5.36. Calcd for $C_{32}H_{30}O_{12} \cdot H_2O$: C, 61.54; H, 5.13%).

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