Phenylphenalenone-Related Compounds: Chemotaxonomic Markers of the Haemodoraceae from *Xiphidium caeruleum*

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Received February 28, 2002

Phytochemical analysis of *Xiphidium caeruleum*, a neotropical member of the family Haemodoraceae, resulted in the isolation and identification of a variety of phenylphenalenone-related compounds. The structures of four new phenylbenzoisochromenones (**3**, **6**, **7**, **10**), a new phenylbenzoisoquinolinone (**4**), and two new oxabenzochrysenones (**11**, **12**) were elucidated using MS and NMR spectroscopic techniques. In addition, five new glucosides (**14**−**18**) were identified, among them four allophanyl glucosides (**15**−**18**), representing a novel type of 6′-substituted glucosidic natural product. On the basis of the common occurrence of these 12 new and four known structures (**2**, **5**, **9**, **13**), hypothetical biosynthetic relationships are discussed. The natural product distribution of other genera of the Haemodoraceae is used as the basis to elaborate biogeographic characteristics of this plant family.

Phenylphenalenones are secondary metabolites of phenylpropanoid origin which have so far been found in the plant families Haemodoraceae,[1] Musaceae,[2] Pontederiaceae,[3] and Strelitziaceae.[4] These compounds are considered chemotaxonomic markers of Haemodoraceae genera such as *Anigozanthos*,[5,6] *Dilatris*,[7] *Haemodorum*,[8] *Lachnanthes*,[9] and *Wachendorfia*.[10] *Xiphidium caeruleum* Aubl. is a neotropical member of the Haemodoraceae native from Mexico to Brazil. So far only one phenylphenalenone, xiphidone (**1**), has been isolated from this species.[11] In this paper we describe the isolation and structure elucidation of 12 new (**3**, **4**, **6**, **7**, **10**−**12**, **14**−**18**) and four known compounds (**2**, **5**, **9**, **13**) from plant material of *X. caeruleum*. The occurrence of phenylphenalenones and related compounds in this species is of considerable interest with respect to chemotaxonomy, biogeography, and phenylpropanoid biogenesis and degradation within the family Haemodoraceae.

Results

Roots, stems, and leaves of whole plants of *Xiphidium caeruleum* were extracted separately with methanol followed by partitioning between *n*-hexane−H_2O, dichloromethane−H_2O, and ethyl acetate−H_2O. The *n*-hexane extract (450 mg) of the root material was used for isolation of nonpolar compounds, whereas the corresponding extracts of stem and leaf material were discarded because of the high chlorophyll content. The dichloromethane (200 mg) and ethyl acetate fractions (150 mg) were employed for isolation of moderately polar compounds and glucosides, respectively, by means of reversed-phase medium-pressure liquid chromatography (MPLC) and reversed-phase HPLC. In continuation of our studies on the occurrence of phenylphenalenones in the plant kingdom,[4,6,12,13] we expected to isolate additional phenylphenalenones from *X. caeruleum*, which could be characterized mainly by NMR and MS methods.

Tetraoxygenated phenylphenalenones and related compounds, modified by oxidative conversions in ring B (lactone structure) or formation of an ether bridge between ring A and ring D, were found (Figure 1), in the latter case resulting in the formation of oxabenzochrysenone-type

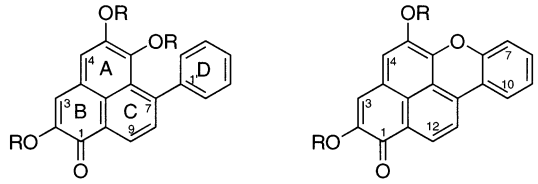

Figure 1. General structures and numbering of phenylphenalenone- and oxabenzochrysenone-type compounds from *X. caeruleum*.

structures. The ^1H NMR spectra of all these compounds were characterized by two singlets of H-3 and H-4 and an AB spin system of H-8/H-9 (or H-11/H-12 in the oxabenzochrysenones, respectively). Proton signals of the exocyclic 7-phenyl ring of phenylphenalenones and a well-resolved four-spin system of H-7 to H-10 in the oxabenzochrysenone-type also gave characteristic resonances. Structural variations were indicated by the additional signals of methoxyl groups, sugar units, and changes in chemical shift values caused by additional oxygen substituents at C-3, for example.

To establish the structures of isolated compounds and to assign ^1H and ^{13}C chemical shifts unambiguously, ^1H NMR and ^{13}C NMR spectra,$^1H-^1H$ COSY, heteronuclear multiple-bond correlation (HMBC), and heteronuclear multiple quantum coherence (HMQC) spectra were measured. The general assigment strategy started from the most downfield signal in the ^1H NMR spectrum, H-9 (phenylphenalenones) or H-12 (oxabenzochrysenone type). These protons exhibited three-bond HMBC correlations with a carbonyl (C-1), C-7 (or 10b, respectively), and the central carbon atom of the tricyclic system, C-9b (12b). The latter carbon atom showed connectivities through three bonds also with H-3 and H-4. The *peri* position of H-3 and H-4 was proved by mutual HMBC cross-peaks with the corresponding carbon atoms C-4 and C-3, respectively. Additional HMBC signals were detected from H-3 with carbonyl C-1 and of H-4 with C-6 (or C-5a in oxabenzochrysenones) through three bonds and C-5 through two bonds. No HMBC correlation was detected between H-3 and C-2 when C-2 was replaced by a heteroatom. In summary, all the isolated compounds gave NMR spectra with similar resonances, which showed the presence of common structural motifs. Structural diversity arose from

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10.1021/np020083s CCC: $22.00
Published on Web 07/11/2002

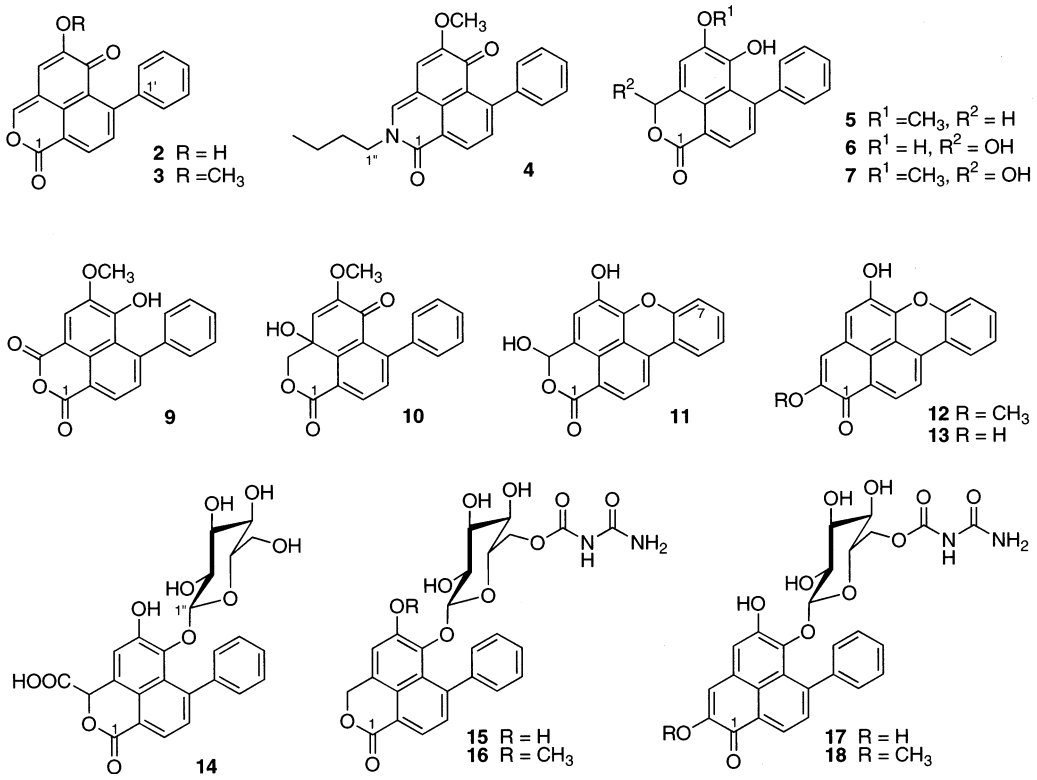

Figure 2. Structures of phenylphenalenone-related natural products from *X. caeruleum*.

Table 1. ^{1}H NMR Data (500 MHz, TMS internal standard) of Compounds **2**−**7**, **9**, and **10**[a]

position	**2** δ_H (J, Hz)	**3** δ_H (J, Hz)	**4** δ_H (J, Hz)	**5** δ_H (J, Hz)	**6** δ_H (J, Hz)	**7** δ_H (J, Hz)	**9** δ_H (J, Hz)	**10** δ_H (J, Hz)
3	8.10 (s)	8.09 (s)	8.01 (s)	5.79 (s)	6.45 (s)	6.85 (s)		4.72 (d, 11.8) 4.59 (d, 11.8)
4	6.98 (s)	6.97 (s)	7.03 (s)	7.55 (s)	7.54 (s)	7.71 (s)	8.32 (s)	6.03 (s)
8	7.68 (d, 8.1)	7.66 (d, 8.1)	7.57 (d, 8.1)	7.36 (d, 7.4)	7.36 (d, 7.4)	7.36 (d, 7.3)	7.52 (d, 7.3)	7.49 (d, 8.0)
9	8.56 (d, 8.1)	8.54 (d, 8.1)	8.64 (d, 8.1)	8.18 (d, 7.4)	8.19 (d, 7.4)	8.23 (d, 7.3)	8.44 (d, 7.3)	8.21 (d, 8.0)
2′/6′	7.36 (m)	7.35 (m)	7.29 (m)	7.37 (m)	7.40 (m)	7.36 (m)	7.41 (m)	7.26 (dd, 7.7, 2.0)
3′/4′/5′	7.43 (m)	7.41 (m)	7.40 (m)					7.40 (m)
OMe-5		3.81 (s)	3.82 (s)	3.98 (s)		4.01 (s)	4.11 (s)	3.71 (s)
1"			4.13 (t, 7.3)					
4"			1.84 (dt, 7.3, 7.6)					
3"			1.46 (dt, 7.6, 7.3)					
4"			1.02 (t, 7.3)					

[a] Compounds **2**, **3**, **5**, **7**, **9**, and **10** were measured in acetone-d_6 and compounds **4** and **6** in methanol-d_4.

the type of substitution at positions 2, 3, 5, and 6. These were elucidated as described below.

The common signals described above were found also in the NMR spectra of 5-hydroxy-7-phenylbenzo[*de*]isochromene-1,6-dione (lachnanthopyrone, **2**) (Figure 2), which was first isolated from flowers of *Lachnanthes tinctoria*.[14] ^{1}H NMR data are shown in Table 1 for comparison purposes. ^{13}C NMR data (Table 2) are published here for the first time.

The NMR data of compound **3** (Tables 1 and 2) closely resembled those of **2**. The presence of the methoxyl group rather than the hydroxyl at position C-5 (δ 152.5) was indicated by the singlet at δ 3.81 and its HMBC correlation with C-5. The structure of compound **3** as 5-methoxy-7-phenylbenzo[*de*]isochromene-1,6-dione was supported by the $[M - 1]^+$ ion (m/z 303) in the EIMS, which is typical for the *peri* position of carbonyl with phenyl. HREIMS confirmed the suggested structure. Compound **3** has been obtained semisynthetically from lachnanthopyrone (**2**) by treatment with diazomethane in ether[14] but hitherto was not known as a natural product.

In addition to ^{1}H and ^{13}C NMR signals very similar to those of compound **3**, the spectra of compound **4** exhibited signals readily assignable to an *n*-butyl chain. HMBC cross-peaks between H_2-1″ (δ 4.13) and C-1 (δ 163.0) and C-3 (δ 138.6) indicated attachment to position 2, so this atom therefore must be a nitrogen. EIMS (m/z 359 $[M]^+$) and HREIMS confirmed compound **4** as an aza-phenylphenalenone, 2-(*n*-butyl)-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione, which is a new natural product. Phenylbenzoisoquinolinones of similar structure (lachnanthopyridones) hitherto have been found only in flowers of *L. tinctoria*.[14,15]

The singlet of H-3 of compound **5** integrated for two protons and therefore represented a methylene group, appearing downfield at δ 5.79, which was consistent with an oxygen function adjacent to C-3. The signal at δ 3.98 indicated a methoxyl at C-5. The hydroxyl group at C-6 was established by means of the acetyl derivative **5a**, which exhibited an acetyl signal at δ 1.39. For ^{1}H NMR data of **5**, see Table 1. The ^{1}H NMR and ^{13}C NMR data of the acetyl derivative **5a** are shown in the Experimental Section. EIMS

Table 2. ^{13}C NMR Data (125 MHz, TMS internal standard) of Compounds **2**−**4**, **6**, **7**, **9**, and **10**[a]

position	**2** δ_C	**3** δ_C	**4** δ_C	**6** δ_C	**7** δ_C	**9** δ_C	**10** δ_C
1	160.6	160.1	163.0	166.4	164.1	162.1	163.6
3	148.8	145.7	138.6	103.8	96.8	161.3	74.7
3a	112.8	111.9	111.4	119.6	122.0	110.0	63.7
4	107.7	104.8	111.3	118.7	114.7	119.6	110.0
5	150.1	152.5	152.8	143.1[b]	144.3	145.3	154.9
6	179.2	177.7	180.3	142.9[b]	143.8	151.5	179.2
6a	125.1	125.6	126.8[b]	119.4	121.4	118.8	125.0
7	152.0	152.0	152.4	146.6	145.5	147.8	148.9
8	134.1	133.6	133.2	129.7	129.7	130.4	133.7[a]
9	134.6	133.8	133.7	127.9	127.7	131.8	133.8[a]
9a	120.6	119.2	125.3[b]	122.6	120.2	120.7	127.7
9b	134.6	133.3	133.3	125.0	125.0	129.6	145.3
1′	142.1	140.7	143.7	144.7	144.5	143.3	142.0
2′,6′	129.1	128.0	129.2	130.0	129.6	129.4	129.1
3′,5′	128.6	127.9	129.1	128.2	127.8	128.1	128.7
4′	128.4	127.8	128.4	129.3	127.5	128.0	128.2
OMe-5		55.7	56.2		57.8	57.7	55.7
1″			50.7				
2″			32.5				
3″			21.0				
4″			14.2				

[a] Compounds **2**, **3**, **7**, **9**, and **10** were measured in acetone-d_6, and compounds **4** and **6** in methanol-d_4. [b] May be reversed within the same column.

(m/z 306 [M$^+$]) and HREIMS of **5** confirmed the structure as 6-hydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one. This compound has been isolated previously from roots of *L. tinctoria*.[9,14]

The chemical shift value of the signal of H-3 (δ 6.45) in compound **6**, which represented one proton (Table 1), was consistent with a hydroxymethine structure at C-3 (δ 103.8) (Table 2). The acetyl derivative **6a** exhibited three acetyl signals (δ 2.29, 2.11, 1.45) from which three hydroxy groups at the positions C-3, C-5, and C-6, respectively, of compound **6** were deduced (for NMR data see Experimental Section). Both LCEIMS (m/z 308 [M$^+$]) and HREIMS data of **6** were in agreement with the suggested structure of 3,5,6-trihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**6**). This compound has been proposed already as an intermediate in the biosynthesis of a naphthalic anhydride and lachnanthopyrone (**2**) in *L. tinctoria*,[9] but hitherto had not been found in any plant.

The ^{1}H NMR spectrum of **7**, representing another new compound, differed from that of the trihydroxy compound **6** only by an additional methoxyl resonance (δ 4.01, Table 1) at C-5 (δ 144.3, Table 2), the position of which was established by a corresponding HMBC cross signal. The LCEIMS data of compound **7** (m/z 322 [M$^+$]) and a pseudomolecular ion m/z 320 [M − 2]$^+$ in the HREIMS indicated dehydrogenation to give an isochromene-1,6-dione or naphthalic anhydride. Diazomethane treatment of compound **7** gave 8-formyl-5,6-dimethoxy-4-phenylnaphthalene-1-carboxylic acid methyl ester (**8**) (EIMS: m/z 350 [M$^+$]) (Figure 3). In addition to the expected introduction of a new methoxyl group in *peri* position to the phenyl ring (δ 3.29/δ 60.7) at C-5 (δ 149.9) the lactone ring was opened, generating a methyl ester and an aldehyde function. The methyl ester gave rise to signals in the NMR spectrum at δ 3.86/δ 52.4 and exhibited an HMBC correlation with the ester carbonyl carbon (δ 169.7). The low-field proton (δ 10.1) and its HMBC cross-peak with C-7 (δ 123.1) and C-8b (δ 124.9) proved the aldehyde functionality.

By means of NMR (Experimental Section) and MS data (EIMS: m/z 320 [M$^+$], HREIMS), 6-hydroxy-5-methoxy-7-phenylbenzo[*de*]isochromene-1,3-dione (**9**) was identified.

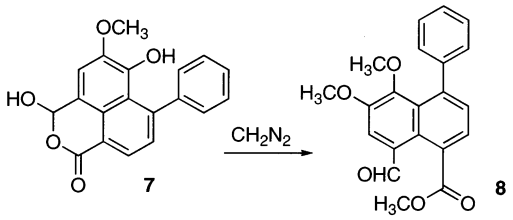

Figure 3. Formation of 8-formyl-5,6-dimethoxy-4-phenylnaphthalene-1-carboxylic acid methyl ester (**8**) from phenylbenzoisochromenone (**7**) by treatment with diazomethane.

This compound was isolated from the *n*-hexane extract of *X. caeruleum* roots and detected in small concentrations in the dichloromethane-soluble fraction of whole plants. Compound **9** seems to be a dehydrogenation product of **7** and has been isolated previously from *L. tinctoria*.[16]

Compound **10** is unusual and distinct from benzoisochromenones **2**−**7** by bearing a tertiary hydroxyl group at C-3a. This was deduced from the chemical shift (δ 63.7) of the ^{13}C NMR signal, which is inconsistent with an aromatic carbon resonance but indicated an oxygenated aliphatic carbon atom. As a consequence of the sp^3 hybridization of C-3a, the conjugated system is interrupted. This showed that C-6 must be a carbonyl carbon, which was confirmed by the ^{13}C NMR chemical shift (δ 179.2) and the C-3 methylene group (δ 74.7). The proton signals of H-3a (δ 4.72) and H-3b (δ 4.59) at the prochiral carbon atom 3 appeared as well-resolved doublets. Attachment of the methoxyl (δ 3.71/δ 55.7) to C-5 (δ 154.9) was established by means of HMBC correlation. EIMS (m/z 322) and HREIMS data were in accord with the structure of **10**, which was fully characterized as a new natural product, 3a-hydroxy-5-methoxy-7-phenyl-3,3a-dihydrobenzo[*de*]isochromene-1,6-dione.

The ^{1}H NMR spectrum of compound **11** showed signals of an AB spin system of H-11 (δ 7.86)/H-12 (δ 8.07) and singlets of H-3 (δ 6.82) and H-4 (δ 7.50). This demonstrated striking analogy with the ^{1}H NMR spectrum of benzo[*de*]-isochromen-1-one (**6**). However, well-resolved signals of a four-spin system were detected rather than the broad phenyl ring signal of compound **6**, indicating replacement of a proton in the *ortho*-position due to substitution or ring closure with the adjacent tricyclic nucleus. HMBC cross signals of H-8 (δ 7.46) and H-10 (δ 8.10) with the low-field-shifted C-6a (δ 153.3) provided evidence for an electronegative substituent in this position. The molecular ion at m/z 306, obtained from EIMS, and HREIMS data proved the molecular formula of $C_{18}H_{10}O_5$, excluding an additional substituent. Thus, a phenol ether bridge existed between H-5a and H-6a. The ^{1}H NMR and MS data discussed here, together with complete ^{13}C NMR data and further HMBC and HMQC connectivities, proved the structure of **11** as 3,5-dihydroxy-3*H*-2,6-dioxabenzo[*def*]chrysen-1-one. This compound represents the first 2,6-dioxabenzochrysenone found as a natural product in plants.

The four-spin system of H-7−H-10 in the ^{1}H NMR spectrum of compound **12** and HMBC connectivities in that part of the molecule resembling compound **11** indicated a structure closely related to **11**. Differences were apparent in positions 2 and 3. Low-field signals of H-3 (δ 7.37) and C-3 (δ 112.7) were inconsistent with sp^3 hybridization but indicated a protonated sp^2 carbon. Consequently, a double bond needed to be accommodated, which is only possible with a carbon at position 2. This carbon atom was detected in the ^{13}C NMR spectrum at δ 152.5 and assigned by a HMBC two-bond correlation with H-3 (δ 7.37) as well as a HMBC three-bond correlation with protons of a methoxyl

Table 3. ^{1}H NMR Data (500 MHz, TMS internal standard) of Compounds **11**−**13**[a]

position	**11** δ_H (*J*, Hz)	**12** δ_H (*J*, Hz)	**13** δ_H (*J*, Hz)
3	6.82 (s)	7.37 (s)	7.36 (s)
4	7.50 (s)	7.79 (s)	7.84 (s)
7	7.22 (dd, 8.2, 1.2)	7.49 (dd, 7.8, 1.2)	7.52 (dd, 8.1, 1.2)
8	7.46 (ddd, 7.6, 8.2, 1.2)	7.62 (ddd, 7.8, 7.5, 1.2)	7.66 (ddd, 8.1, 7.5, 1.2)
9	7.26 (ddd, 7.8, 7.6, 1.2)	7.39 (ddd, 7.5, 8.1, 1.2)	7.45 (ddd, 7.5, 7.8, 1.2)
10	8.10 (dd, 7.8, 1.2)	8.38 (dd, 8.1, 1.2)	8.45 (dd, 7.8, 1.2)
11	7.86 (d, 7.6)	8.31 (d, 7.8)	8.39 (d, 7.7)
12	8.07 (d, 7.6)	8.51 (d, 7.8)	8.73 (d, 7.7)
OMe-2		3.86 (s)	

[a] Compounds **11** and **13** were measured in acetone-d_6 and compound **12** in DMSO-d_6.

Table 4. ^{13}C NMR Data (125 MHz, TMS internal standard) of Compounds **11**−**13**[a]

position	**11** δ_C	**12** δ_C	**13** δ_C
1	163.4	176.8	178.8
2		152.5	150.9
3	97.0	112.7	113.5
3a	119.1	120.1	n.d.[b]
4	119.2	122.0	123.0
5	140.7	140.1	141.2
5a	137.5	137.2	138.4
6a	153.3	151.5	152.8
7	118.4	117.7	118.6
8	132.6	132.3	132.9
9	125.3	124.6	125.4
10	124.8	124.5	125.1
10a	120.5	118.7	119.9
10b	132.4	132.7	134.7
11	115.4	116.1	116.8
12	129.0	130.0	131.5
12a	118.9	119.7	120.5
12b	123.3	117.0	118.2
12c	122.0	127.0	126.8
OMe-2		55.4	

[a] Compounds **11** and **13** were measured in acetone-d_6 and compound **12** in DMSO-d_6. [b] n.d. = not detected due poor signal-to-noise ratio.

group (δ 3.86), which therefore must be attached to C-2. EIMS (*m*/*z* 316 [M$^+$]) and HREIMS confirmed the suggested structure of 5-hydroxy-2-methoxy-6-oxa-benzo[*def*]-chrysen-1-one (**12**), which is a new natural product. Compounds of this type have been described and designated as naphthoxanthenones, first in *L. tinctoria*[9] and afterward in other members of the Haemodoraceae[17,18] and *Musa acuminata*.[18]

The NMR spectra of compound **13** resembled those of **12** except for the absent methoxyl group. Thus, the structure of **13** (EIMS: *m*/*z* 302 [M$^+$]) is 2,5-dihydroxy-6-oxa-benzo[*def*]chrysen-1-one, which has been described already as lachnanthofluorone from roots of *L. tinctoria*.[9]

Five glycosidic compounds (**14**−**18**) were isolated from the ethyl acetate extract of *X. caeruleum*. The aglycon of compound **14** was identified by means of the NMR and MS data (ESIMS: *m*/*z* 499 [M + H]$^+$, HREIMS) as 3-carboxy-5,6-dihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one. The carboxyl group at C-3 (δ 82.2) was established by means of a HMBC correlation between H-3 (δ 6.03) and the carboxyl carbon (δ 174.1). The sugar moiety of **14** was identified as β-D-glucose by means of ^{1}H NMR (Table 5) and ^{13}C NMR data (Table 6) and a complete set of HMBC and HMQC correlations. The doublet of H-1″ at δ 4.75 showed a coupling constant of 7.9 Hz, indicating the β-configuration of the anomeric proton. The coupling constants $^3J_{H-2-H-3}$, $^3J_{H-3-H-4,}$ and $^3J_{H-4-H-5}$ of around 9 Hz showed that all protons are axial. The HMBC correlation between H-1″ and C-6 (δ 139.0) established attachment of the glucose unit to C-6 of the aglycon. Mutual interactions between the aromatic ring and the glucose unit in the *peri* position were observed. Due to the shielding effect of the phenyl ring, the double doublets of H-2″ (δ 2.39) and H-4″ (δ 2.97) appeared at unusually high field. Similar high-field shifts in glucosides[19] have been observed already for such protons of the antibiotic moenomycin A[20] and benzoxazinone-*N*-glucosides.[21] Notably, H-2″ of glucoside **14** was drastically shifted to high field, while the corresponding carbon signals of C-2″ (δ 74.7) and C-4″ (δ 71.4) appeared within the expected usual shift range. Electronic effects of the chiral sugar unit, which is in close proximity to the phenyl ring, caused significant signal broadening. Moreover, separate signals of magnetically inequivalent pairs of protons H-2′ (δ 7.31)/H-6′ (δ 7.35), H-3′ (δ 7.48)/H-5′ (δ 7.26) and carbon atoms C-2′ (δ 127.3)/C-6′ (δ 128.1) and C-3′ (δ 130.6)/C-5′ (δ 131.4) were observed. From these data, the structure of compound **14** was shown to be 3-carboxy-5-hydroxy-6-*O*-β-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one. However, attempts to hydrolyze this new glucoside by means of β-glucosidase were not successful. This is in accord with reported resistance to various glycosidases of the 1-*O*-glucoside of haemocorin aglycon,[7] which very likely is due to the steric hindrance of a phenyl *peri* to the bulky sugar unit.

The aglycon of glycoside **15** was identified by means of the NMR data as 5-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one. It differed from that of **14** only in the absence of the carboxyl group at C-3 (δ 70.7), which was indicated by the methylene singlet of H-3 at δ 5.75. Again, separate signals of magnetically inequivalent pairs of phenyl protons and phenyl carbon atoms were observed, together with the HMBC correlation of H-1″ (δ 4.60) with C-6 (δ 138.4), which proved the attachment of the hexose unit to this carbon atom. This sugar moiety was identified as β-D-glucose by ^{1}H NMR, ^{13}C NMR, and HMBC signals. However, two further low-field ^{13}C NMR signals at δ 172.4 and 170.0, the latter being connected to H-6″ (δ 4.16/3.91) of the glucose unit by HMBC, suggested a two-carbon substituent at C-6″-OH. The mass spectrum of **15** (ES-IMS: *m*/*z* 541 [M + 1]$^+$, HREIMS) indicated the molecular formula $C_{26}H_{24}N_2O_{11}$. After identification of the aglycon and the glucose moiety, a $C_2H_3N_2O_2$ substituent was suggested, indicating an (aminocarbonyl)carbamic acid (allophanyl) unit. However, owing to the instability of allophanic acid,[22] a fragment of an intact allophanyl unit was not detectable in the mass spectrum. To confirm the allophanyl substituent, compound **15** was transesterified by sodium methylate and analyzed by means of MS. The molecular ion (EIMS: *m*/*z* 118 [M]$^+$) and fragmentation pattern of the resulting methyl ester were the same as those of methyl allophanate, obtained by transesterification of commercially available ethylallophanate. HREIMS confirmed the structure as well. Compound **15**, 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-

Table 5. ^{1}H NMR Data (500 MHz, methanol-d_4, TMS internal standard) of Glucosides **14**−**18**

position	**14** δ_H (J, Hz)	**15** δ_H (J, Hz)	**16** δ_H (J, Hz)	**17** δ_H (J, Hz)	**18** δ_H (J, Hz)
3	6.03 (s)	5.75 (s)	5.79 (s)	7.04 (s)	7.13 (s)
4	7.48 (s)	7.22 (s)	7.46 (s)	7.46 (s)	7.57 (s)
8	7.37 (d, 7.6)	7.40 (d, 7.4)	7.35 (d, 7.4)	7.54 (d, 7.6)	7.56 (d, 7.5)
9	8.14 (d, 7.6)	8.15 (d, 7.4)	8.12 (d, 7.4)	8.47 (d, 7.6)	8.48 (d, 7.5)
2′	7.31 (br)	7.32 (br)	7.33 (br)	7.33 (br)	7.33 (br)
3′	7.48 (br)	7.60 (br)	7.46 (br)	7.43 (br)	7.46 (br)
4′	7.31 (br)	7.32 (br)	7.33 (br)	7.33 (br)	7.35 (br)
5′	7.26 (br)	7.20 (br)	7.20 (br)	7.22 (br)	7.23 (br)
6′	7.35 (br)	7.37 (br)	7.41 (br)	7.39 (br)	7.40 (br)
1″	4.75 (d, 7.9)	4.60 (d, 7.9)	4.82 (d, 7.7)	4.67 (d, 7.9)	4.66 (d, 7.7)
2″	2.39 (dd, 7.9, 9.3)	2.35 (dd, 7.9, 9.3)	2.07 (dd, 7.7, 9.1)	2.36 (dd, 7.9, 9.0)	2.37 (dd, 7.7, 8.9)
3″	3.18 (dd, 9.3, 9.5)	3.17 (dd, 9.3, 8.4)	3.17 (dd, 9.1, 8.8)	3.19 (dd, 9.0, 9.3)	3.19 (dd, 8.9, 9.0)
4″	2.97 (dd, 9.5, 9.8)	3.05 (m)	3.01 (dd, 8.8, 9.7)	3.04 (dd, 9.3, 9.0)	3.00 (dd, 9.0, 9.2)
5″	2.92 (ddd, 9.8, 5.3, 2.2)		4.27 (ddd, 9.7, 5.4, 2.0)	3.09 (ddd, 9.0, 6.5, 2.2)	3.10 (ddd, 9.2, 6.6, 2.0)
6″a	3.47 (dd, 11.8, 5.3)	4.16 (dd, 11.5, 4.8)	4.21 (dd, 11.5, 5.4)	4.18 (dd, 11.8, 6.5)	4.17 (dd, 11.8, 6.6)
6″b	3.57 (dd, 11.8, 2.2)	3.91 (dd, 11.5, 2.0)	3.93 (dd, 11.5, 2.0)	3.96 (dd, 11.8, 2.2)	4.00 (dd, 11.8, 2.0)
OMe-2					3.94 (s)
OMe-5			3.96 (s)		

Table 6. ^{13}C NMR Data (125 MHz, methanol-d_4, TMS internal standard) of Glucosides **14**−**18**

position	**14** δ_C	**15** δ_C	**16** δ_C	**17** δ_C	**18** δ_C
1	167.1	166.9	166.7	182.2	181.9
2				151.2	153.6
3	82.2	70.7	70.8	116.3	115.0
3a	126.2	126.4	126.0	128.5	129.0[a]
4	118.6	116.6	112.9	124.2	124.6
5	148.6	148.9	151.0	149.0	149.0
6	139.0	138.4	139.9	141.0	141.3
6a	121.2	120.0	120.0	127.8[a]	127.4[a]
7	145.5	146.1	146.6	148.8	148.7
8	131.7	131.8	131.8	132.3	132.4
9	126.5	127.0	127.6	128.6	128.8
9a	128.4	128.9	129.9	130.0[a]	129.1[a]
9b	125.5	125.8	126.0	121.7	121.8
1′	145.1	144.9	145.2	145.3	145.1
2′	127.3 (br)	127.3 (br)	127.3 (br)	127.3 (br)	127.4 (br)
3′	130.6 (br)	130.9 (br)	130.6 (br)	130.7 (br)	130.7 (br)
4′	127.6	127.8	127.6	127.8	127.8
5′	131.4 (br)	131.6 (br)	131.3 (br)	131.8 (br)	131.9 (br)
6′	128.1 (br)	128.2 (br)	127.6 (br)	128.3 (br)	128.2 (br)
1″	103.9	104.0	102.9	104.1	104.1
2″	74.7	74.6	74.2	74.6	74.5
3″	77.6	77.5	77.4	77.6	77.6
4″	71.4	71.1	71.2	71.1	71.3
5″	77.8	75.1	74.6	75.2	75.0
6″	62.7	64.8	65.3	64.9	65.2
1‴		170.0	168.0	169.3	168.2
2‴		172.4	169.7	171.5	169.9
COOH-3	174.1				
OMe-2					56.1
OMe-5			57.5		

[a] May be reversed within the same column.

5-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one, is the first allophanylglucoside detected as a natural product. Derivatives of allophanic acid hitherto have been found only in two plant species, *Butea monospermae*[23,24] and *Echinops echinatus*.[25]

Compound **16** gave spectra that were similar to **15** with the exception of the additional methoxyl signal at position C-5 (δ 151.0). The aglucon of **16** was readily assigned by means of NMR data (Table 5, Table 6) as compound **5**. The NMR spectra of the acetyl derivative **16a** (see Experimental Section) exhibited additional signals for three acetyl groups of the glucose unit, confirming substitution by the allophanyl moiety. Mass spectra (ESIMS: m/z 555 $[M + H]^+$, HREIMS) confirmed the structure of **16** as 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one.

Signals of the allophanylglucosyl moiety were also found in the NMR spectra of compound **17**. An HMBC correlation of H-1″ (δ 4.67) with C-6 (δ 141.0) proved the attachment of the glucose to the aglycon at this position. The ^{1}H NMR spectrum (Table 5) exhibited aglycon signals, namely, two singlets of H-4 (δ 7.46) and H-3 (δ 7.04), the AB spin system of H-8 (δ 7.54)/H-9 (δ 8.47), and signals of a phenyl group. The chemical shift of H-3 and an additional HMBC correlation through two bonds with the carbon signal at δ 151.2 (Table 6) were consistent with a hydroxyl group bearing carbon in position 2. In analogy with allophanylglucosides **15** and **16**, separate signals of magnetically inequivalent pairs of protons and carbon atoms were observed in the ^{1}H and ^{13}C NMR spectra of compound **17**. This phenomenon can be considered indicative of close spatial proximity of the phenyl ring with the allophanylglucose unit in *peri* position. The molecular weight of **17** (ESIMS: m/z 553 $[M + H]^+$, HREIMS) confirmed these findings and demonstrated compound **17** to be 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-2,5-dihydroxy-7-phenylphenalen-1-one. The aglycon of **17** is hitherto not known as a natural product, although similar compounds such as haemodorin (**20**)[26] and xiphidone (**1**)[11] have been previously isolated from *Haemodorum distichophyllum* and *X. caeruleum*, respectively.

NMR data of compound **18** resembled those of **17** but with an additional methoxyl signal (δ 3.94/δ 56.1), which exhibited a HMBC correlation through three bonds with the carbon signal of C-2 (δ 153.6). On the basis of these assignments, compound **17** is 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-hydroxy-2-methoxy-7-phenylphenalen-1-one. The aglycons of compounds **17** and **18**, except the previously described compound **1**, represent the only intact phenylphenalenones found in *X. caeruleum*.

A significant number of natural products isolated from *X. caeruleum* bear methoxyl substituents. This finding raised the question of artificial methylation during an extraction procedure using methanol as a solvent. To check this possibility, control experiments were carried out using acetone rather than methanol as the solvent for extraction. HPLC analysis with a MeCN−H_2O gradient indicated the occurrence of all compounds described above, confirming that these metabolites are indeed natural products. However, three additional compounds, namely, 5,6-dihydroxy-3-methoxy-, 5-hydroxy-3,6-dimethoxy-, and 6-hydroxy-3,5-dimethoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-ones, representing methoxy analogues of **6** and **7**, respectively, were

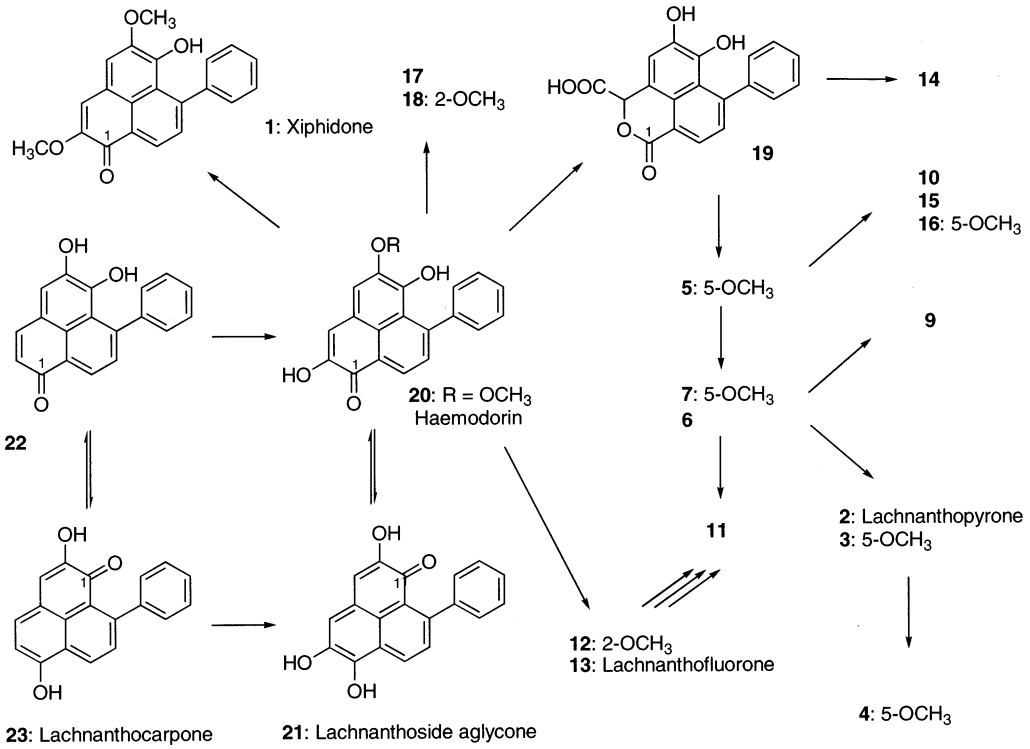

Figure 4. Proposed biosynthetic relationships between phenylphenalenone-related compounds from the subfamily Haemodoroideae of the Haemodoraceae. Compounds **20**−**23** have not been found so far in *X. caeruleum*. Compound **19** represents the aglycon of glycoside **14** and was not found in free form. The variable methylation pattern can be explained by parallel pathways involving either methylated or unmethylated compounds.

found after extraction with methanol (data not shown). In these experiments, the HPLC peaks representing compound **6** are significantly diminished compared with those from acetone extraction. Moreover, 3-ethoxy-6-hydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one was found as an additional compound when ethanol was used for extraction. These findings clearly indicated that the methoxyl group at C-3 of benzoisochromenones is of artificial origin.

Discussion

The C-19 phenylphenalenone carbon skeleton is biosynthesized from two phenylpropanoid units and C-2 from acetate.[27,28] Although not having been studied in detail, oxygen functions at positions 1 and 6 of the phenylphenalenone-type structure shown in Figure 1 are suggested to originate from the carboxyl and *p*-hydroxyl groups of the phenylpropanoid precursor, *p*-coumaric acid. In contrast, further oxygenations at the ring system are considered to occur later in the biosynthesis. The biosynthetic origin of the oxalactone structure in ring B, which is a structural feature of the majority of natural products isolated so far from *X. caeruleum*, has not yet been investigated. Intact phenylphenalenones possessing a C-19 skeleton (**20**−**23**) (Figure 4) are assumed to function as precursors for the entire family of related structures described here. The finding that phenylbenzoisochromenones (phenylnaphthalides) and other oxalactone compounds represent the most abundant natural products of *X. caeruleum* suggests that oxidative metabolism of phenalenones seems to be not a spontaneous process but is enzymatically catalyzed, at least in the initial step. Oxidation of **22** next to the carbonyl function, at C-2, might be the first step in this hypothetical biosynthetic sequence (Figure 4). This is substantiated by the common occurrence of trioxygenated phenylphenalenones, such as lachnanthocarpone (**23**), and tetraoxygenated compounds of that type, for example, lachnanthoside aglycon (**21**) in *Lachnanthes tinctoria*[9,29] and *Wachendorfia paniculata*.[10] Oxidative rearrangement of tautomeric tetraoxygenated phenylphenalenones **20**/**21** would result in the formation of 3-carboxyoxalactone-type compounds such as compound **19**. Further steps, namely, decarboxylation and hydroxylation at C-3, would deliver compound **7**, which upon further conversion by dehydration and dehydrogenation, would give lachnanthopyrone (**2**) and the anhydride **9**, respectively. A part of the pathway has already been proposed.[9] The hypothetical biosynthetic scheme, shown in Figure 4, also accounts for the additional compounds isolated from *X. caeruleum*. A phenylphenalenone-derived alkaloid, the phenylbenzoisoquinoline-1,6-dione (**4**), could be formed from compound **3**. Starting from compound **20**, a parallel oxidative pathway via oxabenzochrysenone-type compounds (e.g., lachnanthofluorone, **13**) to the dioxabenzochrysenone **11** seems to be operational in *X. caeruleum*. Alternatively, **11** may be formed from compound **7** after oxidation of ring B. Since in several cases 5-hydroxy and 5-methoxy compounds coexist, the occurrence of both complete series can be predicted.

Interestingly, glucosides were found only with sugars in the sterically hindered position *peri* to the phenyl ring. The consequences on the NMR spectra have been discussed above. Owing to lack of a hydroxyl in such a position in oxabenzochrysenones, glucoside formation obviously does not take place with these compounds in *X. caeruleum*. The allophanylglucosides represent a novel type of conjugate. Their role in plants is still completely unknown and has to be investigated.

The occurrence of members of the same subgroup of phenylphenalenone-related compounds, such as phenylbenzoisochromenones, phenylbenzoisoquinolinediones, and even identical compounds of these types (e.g., **2** and **5**) in *X. caeruleum* and the North American Haemodoraceae species, *L. tinctoria*, represents a strong argument for a very close relationship between both species. These chemo-

taxonomic considerations indicate that these structural types might be characteristic features of the Western hemisphere Haemodoraceae belonging to the subfamily Haemodoroideae[30] (although there are published no investigations on the genera *Schiekia* and *Pyrrorhiza* from South America). American species seem to contain no phenylphenalenones with a completely deoxygenated B-ring (like 9-phenylphenalenones such as anigorufone and hydroxyanigorufone), which are characteristic compounds of Australian genera of the subfamily Conostylidoideae, especially *Anigozanthos*.[6,31] *Wachendorfia*, a South African genus of the subfamily Haemodoroideae, seems to occupy an intermediate position since both types, phenylbenzoisochromenones and 9-phenylphenalenones, were identified in *W. thyrsiflora* (Opitz and Schneider, unpublished data).

Our results support the hypothesis of a Gondwanan origin of the Haemodoraceae based on biogeographic distribution pattern and morphometric studies.[32] Thus, American species would be more closely related to South African than to Australian ones because of the mutual drift of recent South America and Africa apart from Antarctica and Australia. However molecular phylogenetic analysis has revealed a possibly much older origin with a largely relictal distribution.[33]

In summary, phytochemical data presented here contribute to the establishment of relationships within the plant family Haemodoraceae. Further phytochemical work and, in addition, genetic analyses are required for final clarification of evolutionary origin and development of biosynthetic pathways of this family.

Experimental Section

General Experimental Procedures. NMR spectra were measured on a Bruker DRX 500 NMR spectrometer, operating at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C using standard Bruker pulse sequences. Chemicals shifts are given in δ values referring to TMS as internal standard. ^{1}H NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments were recorded in a 2.5 mm inverse detection microprobe head; broadband decoupled ^{13}C spectra were run using a 2.5 mm broadband microprobe head.

Electron-impact mass spectra (EIMS) and high-resolution spectra (HREIMS) were recorded on a MasSpec sector field mass spectrometer (Micromass Ltd., Manchester, UK) with a direct insertion probe. Electrospray ionization mass spectra (ESIMS) were recorded on a Micromass Quattro II tandem quadrupole mass spectrometer using the first quadrupole only. For LC-MS analysis, a Hewlett-Packard HP 1100 LC was connected to the Quattro II (flow rate 1 mL min^{-1}). HRESIMS were recorded on a Quattro II or Micromass LCT mass spectrometer (fitted with a "LockSpray" source) at a resolution of 5000.

UV spectra were obtained from an Agilent G1315B diode array detector during analytical HPLC in MeCN−H_2O. Preparative HPLC was conducted on a Merck Hitachi LiChrograph chromatography system (L-6200A gradient pump, L-4250 UV−vis detector) using a Nucleosil 100-7 C_{18} (7 μm; 250 × 21 mm) and a LiChrospher 100 RP18 column (10 μm; 250 × 10 mm). Analytical HPLC was carried out on a Agilent 1100 chromatography system (binary pump G1312A, DAD G1315B, autosampler G1313A) using a LiChrospher 100 RP-18 column (5 μm; 250 × 4 mm). Medium-pressure liquid chromatography (MPLC) was conducted using a Büchi B-688 pump equipped with a LiChroprep RP18 column (25−40 μm, 230 × 22 mm). TLC was performed on silica gel 60 F_{254} using precoated plates and 2:1 toluene−acetone as eluent. Compounds on TLC plates were detected from their visible absorbance.

Plant Material. Plants of *Xiphidium caeruleum* Aubl. were obtained from the University of Bochum (Botanical Institute), vegetatively propagated, and maintained at the Botanical Garden of the University of Jena. The plants were transferred to the Max Planck Institute for Chemical Ecology, Jena, and, prior to extraction, held in growth chambers for variable time intervals at a minimum temperature of 15 °C. Three-year-old plants were used in the experiments.

Extraction and Isolation. Root (90 g), stem (320 g), and leaf (450 g) material of whole plants were frozen with liquid N_2, ground, and extracted exhaustively with MeOH or acetone at room temperature. After evaporation (<40 °C) the extracts were partitioned between *n*-hexane−H_2O, CH_2Cl_2−H_2O, and EtOAc−H_2O. The *n*-hexane extract was used only from the roots and separated by TLC on silica gel 60 F_{254} (0.5 mm layer thickness) using toluene−acetone (2:1). The major colored TLC zone (R_f 0.6) was scraped off, eluted with EtOAc, passed through a RP-18 cartridge (elution with acetone), and finally purified by means of reversed-phase HPLC on a LiChrospher 100 RP-18 (5 μm; 250 × 4 mm), using linear gradient (a) MeCN−H_2O, 3:7 → 13:7 in 30 min, then raised to 9:1 in another 5 min or (b) MeCN−H_2O, 1:1 → 1:0 in 30 min (flow rate 0.8 mL min^{-1}; DAD detection 200−600 nm).

The CH_2Cl_2 and EtOAc extracts, respectively, from the roots, stems, and leaves were pooled, subjected to MPLC, and stepwise eluted using mixtures of MeCN−H_2O, 1:4, 3:7, 2:3, 1:1, 3:2, 7:3, 4:1 (100−500 mL). Fractions eluting with 2:3 and 1:1 MeCN−H_2O were collected and evaporated to dryness. Further separation was carried out by preparative reversed-phase HPLC on Nucleosil 100-7 C_{18} (7 μm; 250 × 21 mm; flow rate 11.5 mL min^{-1}; UV 254 nm) or LiChrospher 100 RP18 (10 μm; 250 × 10 mm; flow rate 3.5 mL min^{-1}; UV 254 nm). Gradient (a) was used also for the CH_2Cl_2 fraction and (c) 1:19 → 1:1 MeCN−H_2O (0.1% TFA) in 40 min for the EtOAc fraction. Final purification was performed by reversed-phase HPLC on LiChrospher 100 RP-18 (5 μm; 250 × 4 mm) using the same gradients as with the large columns (flow rate 0.8 mL min^{-1}, DAD detection 200−600 nm).

Derivatization. Acetylation was performed by means of acetic anhydride in pyridine. Diazomethane in ether was used for methylation. Transesterification of allophanylglucoside **15** to methylallophanate (EIMS *m/z* 118 [M$^+$] (20), 87 (5), 75 (79), 70 (5), 59 (9), 44 (100); HREIMS *m/z* 118.0378 (calcd for $C_3H_6N_2O_3$, 118.0378) was performed using sodium methylate. Methylallophanate was prepared for reference purposes from commercially available ethylallophanate using the same procedure.

5-Hydroxy-7-phenylbenzo[*de*]isochromene-1,6-dione (lachnanthopyrone, 2): HPLC gradient (a) t_R 23.0 min, isolated amount 1.5 mg; UV (MeCN−H_2O) λ_{max} 243, 395 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 289 [M − 1]$^+$ (100); HREIMS *m/z* 290.0571 (calcd for $C_{18}H_{10}O_4$, 290.0579).

5-Methoxy-7-phenylbenzo[*de*]isochromene-1,6-dione (3): HPLC gradient (a) t_R 22.6 min, isolated amount 1.6 mg; UV (MeCN−H_2O) λ_{max} 243, 390 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 303 [M − 1]$^+$ (100); HREIMS *m/z* 303.9850 (calcd for $C_{19}H_{12}O_4$, 303.9855).

2-(*n*-Butyl)-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (4): HPLC gradient (a) t_R 30.5 min, isolated amount 0.6 mg; UV (MeCN−H_2O) λ_{max} 238, 320, 334 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 359 [M]$^+$ (100), 358 (96), 330 (14), 302 (28), 272 (18); HREIMS *m/z* 359.1528 (calcd for $C_{23}H_{21}NO_3$, 359.1521).

6-Hydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (5): HPLC gradient (a) t_R 24.8 min, isolated amount 1.5 mg; UV (MeCN−H_2O) λ_{max} 214, 268, 388, nm; ^{1}H NMR data, see Table 1; EIMS *m/z* 306 [M]$^+$ (100); HREIMS *m/z* 306.0883 (calcd for $C_{19}H_{14}O_4$, 306.0892).

6-Acetoxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (5a): UV (MeCN−H_2O) λ_{max} 216, 255, 328, 360 nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.21 (1H, d, J = 7.3 Hz, H-9), 7.67 (1H, s, H-4), 7.47 (3H, m, H-3′,5′ and H-4′), 7.44 (1H, d, J = 7.3 Hz, H-8), 7.35 (2H, m, H-2′,6′), 5.90 (2H, s, H-3), 3.94 (3H, s, OCH_3-5), 1.39 (3H, s, OCOCH_3-6); ^{13}C NMR (acetone-d_6, 125 MHz) δ 168.7 (O*C*OCH$_3$-6), 163.9 (C-1), 150.6 (C-5), 143.3, 143.2 (C-7 and C-1′), 134.0 (C-6), 131.5 (C-8), 129.6 (C-3a), 128.6 (br, C-2′,6′), 128.3 (C-4′), 128.0 (C-3′,5′), 126.7 (C-9), 125.9 (C-6a), 125.4 (C-9b), 121.1 (C-9a), 111.9 (C-4), 70.1

(C-3), 57.2 (O*C*H$_3$-5), 19.4 (OCO*C*H$_3$-6); EIMS *m/z* 348 [M]$^+$ (37), 306 (100), 289 (17); HREIMS *m/z* 348.0993 (calcd for $C_{21}H_{16}O_5$, 348.0998).

3,5,6-Trihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (6): HPLC gradient (a) t_R 13.3 min, isolated amount 2.0 mg; UV (MeCN−H$_2$O) λ_{max} 269, 341, 387 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LCEIMS *m/z* 308 [M]$^+$; HRESIMS *m/z* 309.0771 [M + H]$^+$ (calcd for $C_{18}H_{13}O_5$, 309.0763).

3,5,6-Triacetoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (6a): UV (MeCN−H$_2$O) λ_{max} 209, 144, 326 nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.49 (1H, d, J = 7.7 Hz, H-9), 7.87 (1H, s, H-4), 7.80 (1H, s, H-3), 7.64 (1H, d, J = 7.7 Hz, H-8), 7.52 (3H, m, H-3′,5′ and H-4′), 7.39 (2H, m, H-2′,6′), 2.29 (3H, s, OCOC*H*$_3$-5), 2.11 (3H, s, OCOC*H*$_3$-3), 1.45 (3H, s, OCOC*H*$_3$-6); ^{13}C NMR (acetone-d_6, 125 MHz) δ 169.3 (O*C*OCH$_3$-3), 168.7 (O*C*OCH$_3$-5), 168.4 (O*C*OCH$_3$-6), 162.0 (C-1), 145.4 (C-7), 142.3 (C-5), 142.3 (C-1′), 140.2 (C-6), 132.1 (C-8), 130.4 (C-9), 129.7 (br, C-2′,6′), 128.8 (C-3a), 128.8 (C-3′,5′), 128.6 (C-4′), 128.0 (C-9b), 125.7 (C-9a), 124.1 (C-4), 119.8 (C-6a), 92.3 (C-3), 20.8 (OCO*C*H$_3$-3), 20.5 (OCO*C*H$_3$-5), 19.3 (OCO*C*H$_3$-6); EIMS *m/z* 434 [M]$^+$ (20), 392 (35), 350 (82), 291 (100); HREIMS *m/z* 434.1008 (calcd for $C_{24}H_{18}O_8$, 434.1002).

3,6-Dihydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromene-1-one (7): HPLC gradient (a) t_R 18.0 min, isolated amount 2.5 mg; UV (MeCN−H$_2$O) λ_{max} 266, 340, 386 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LCEIMS *m/z* 322 [M]$^+$ (60), 303 (100), 273 (37), 176 (61); EIMS *m/z* 320 [M$^+$] (26), 303 (100); HREIMS *m/z* 320.0695 (calcd for $C_{19}H_{12}O_5$, 320.0685).

8-Formyl-5,6-dimethoxy-4-phenylnaphthalene-1-carboxylic acid methyl ester (8): UV (MeCN−H$_2$O) λ_{max} 208, 244, 364 nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 10.12 (1H, s, C*H*O), 8.13 (1H, s, H-7), 7.89 (1H, d, J = 7.3 Hz, H-2), 7.35 (1H, d, J = 7.3 Hz, H-3), 7.32−7.41 (6H, m, H-3, H-2′−H-6′), 4.06 (3H, s, OC*H*$_3$-6), 3.86 (3H, s, COOC*H*$_3$), 3.29 (3H, s, OC*H*$_3$-5); ^{13}C NMR (acetone-d_6, 125 MHz) δ 190.7 (*C*HO), 169.7 (C-1), 150.1 (C-6), 149.9 (C-5), 145.2 (C-1′), 143.2 (C-4), 131.2 (C-8), 130.3 (C-3), 129.0 (C-2′,6′), 129.1 (C-4a), 128.8 (C-2), 127.9 (C-3′,5′), 127.1 (C-4′), 124.9 (C-8a), 123.1 (C-7), 60.7 (O*C*H$_3$-5), 57.2 (O*C*H$_3$-6), 52.4 (COO*C*H$_3$); EIMS *m/z* 350 [M]$^+$ (100), 334 (35), 322 (47), 319 (23), 291 (20), 275 (25), 248 (22); HREIMS *m/z* 350.1139 (calcd for $C_{21}H_{18}O_5$, 350.1154).

6-Hydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromene-1,3-dione (9): HPLC gradient (b) t_R 26.3 min, isolated amount 7.6 mg; UV (MeCN−H$_2$O) λ_{max} 209, 267, 347, 413 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 320 [M]$^+$ (100), 276 (7), 261 (21), 233 (12), 176 (12); HREIMS *m/z* 320.0683 (calcd for $C_{19}H_{12}O_5$, 320.0685).

3a-Hydroxy-5-methoxy-7-phenyl-3,3a-dihydrobenzo-[*de*]isochromene-1,6-dione (10): HPLC gradient (a) t_R 13.0 min, isolated amount 1.0 mg; UV (MeCN−H$_2$O) λ_{max} 200, 232, 296 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 322 [M]$^+$ (18), 306 (60), 303 (100), 292 (37), 289 (30), 263 (29); HREIMS *m/z* 322.0885 (calcd for $C_{19}H_{14}O_5$, 322.0841).

3,5-Dihydroxy-3*H*-2,6-dioxabenzo[*def*]chrysen-1-one (11): HPLC gradient (a) t_R 13.9 min, isolated amount 0.8 mg; UV (MeCN−H$_2$O) λ_{max} 230, 269, 435 nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; EIMS *m/z* 306 [M]$^+$ (100); HREIMS *m/z* 306.0516 (calcd for $C_{18}H_{10}O_5$, 306.0528).

5-Hydroxy-2-methoxy-6-oxabenzo[*def*]chrysen-1-one (12): HPLC gradient (a) t_R 20.4 min, isolated amount 0.8 mg; UV (MeCN−H$_2$O) λ_{max} 237, 320, 386, 547 nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; EIMS *m/z* 316 [M]$^+$ (100); HREIMS *m/z* 316.0734 (calcd for $C_{20}H_{12}O_4$, 316.0736).

2,5-Dihydroxy-6-oxabenzo[*def*]chrysen-1-one (13): HPLC gradient (a) t_R 24.0 min, isolated amount 0.5 mg; UV (MeCN−H$_2$O) λ_{max} 238, 320, 396, 553 nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; EIMS *m/z* 302 [M]$^+$ (100); HREIMS *m/z* 302.0581 (calcd for $C_{19}H_{10}O_4$, 302.0579).

3-Carboxy-5-hydroxy-6-*O*-β-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (14): HPLC gradient (c)-t_R 24.9 min, isolated amount 4 mg; UV (MeCN−H$_2$O) λ_{max} 221, 260, 334, 367 nm; ^{1}H NMR data, see Table 5; ^{13}C NMR data, see Table 6; ESIMS *m/z* 499 [M + H]$^+$ (5), 337 (100); HRESIMS *m/z* 499.1263 [M + H]$^+$ (calcd for $C_{25}H_{23}O_{11}$, 499.1240).

6-*O*-[(6″-*O*-Allophanyl)-β-D-glucopyranosyl]-5-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (15): HPLC gradient (c) t_R 26.3 min, isolated amount 1.5 mg; UV (MeCN−H$_2$O) λ_{max} 214, 260, 334, 366 nm; ^{1}H NMR data, see Table 5; ^{13}C NMR data, see Table 6; ESIMS *m/z* 541 [M + H]$^+$ (18), 293 (100), 231 (9); HRESIMS *m/z* 541.1378 [M + H]$^+$ (calcd for $C_{26}H_{25}N_2O_{11}$, 541.1458).

6-*O*-[(6″-*O*-Allophanyl)-β-D-glucopyranosyl]-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (16): HPLC gradient (c) t_R 26.6 min, isolated amount 2.5 mg; UV (MeCN−H$_2$O) λ_{max} 199, 260, 336, 370 nm; ^{1}H NMR data, see Table 5; ^{13}C NMR data, see Table 6; ESIMS *m/z* 555 [M + H]$^+$ (4), 307 (100), 231 (5); HRESIMS *m/z* 555.1659 [M + H]$^+$ (calcd for $C_{27}H_{27}N_2O_{11}$, 555.1615).

6-*O*-[(2″,3″,4″-Tri-*O*-acetyl-6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (16a): UV (MeCN−H$_2$O) λ_{max} 214, 258, 334, 364 nm; ^{1}H NMR (acetone-d_6, 500 MHz) 8.21 (1H, d, J = 7.4 Hz, H-9), 7.63 (1H, s, H-4), 7.47 (1H, d, J = 7.4 Hz, H-8), 7.30−7.55 (5H, brm, H-2′−H-6′), 5.86 (2H, s, H-3), 5.17 (1H, d, J = 7.8 Hz, H-1″), 5.11 (1H, dd, J = 9.6, 9.6 Hz, H-3″), 4.66 (1H, dd, J = 9.6, 9.6 Hz, H-4″), 4.20 (1H, dd, J = 12.1, 5.9 Hz, H-6″a), 4.03 (3H, s, OC*H*$_3$-5), 3.96 (1H, dd, J = 7.8, 9.6 Hz, H-2″), 3.75 (1H, dd, J = 12.1, 2.6 Hz, H-6″b), 3.59 (1H, ddd, J = 9.6, 5.9, 2.6 Hz, H-5″), 1.97 (3H, COOC*H*$_3$), 1.95 (3H, COOC*H*$_3$), 1.93 (3H, COOC*H*$_3$); ^{13}C NMR (acetone-d_6, 125 MHz) δ 127.0 (C-9), 131.3 (C-8), 112.3 (C-4), 100.5 (C-1″), 72.9 (C-3″), 72.3 (C-2″), 71.5 (C-5″), 69.8 (C-3), 68.9 (C-4″), 63.4 (C-6″), 57.3 (O*C*H$_3$-5), 20.6 (COO*C*H$_3$), 20.5 (COO*C*H$_3$), 20.5 (COO*C*H$_3$).

6-*O*-[(6″-*O*-Allophanyl)-β-D-glucopyranosyl]-2,5-dihydroxy-7-phenylphenalen-1-one (17): HPLC gradient (c) t_R 26.2 min, isolated amount 0.9 mg; UV (MeCN−H$_2$O) λ_{max} 211, 279, 378, 477 nm; ^{1}H NMR data, see Table 5; ^{13}C NMR data, see Table 6; ESIMS *m/z* 553 [M + H]$^+$ (4), 305 (100); HRESIMS *m/z* 575.1286 [M + Na]$^+$ (calcd for $C_{27}H_{24}N_2O_{11}Na$, 575.1278).

6-*O*-[(6″-*O*-Allophanyl)-β-D-glucopyranosyl]-5-hydroxy-2-methoxy-7-phenylphenalen-1-one (18): HPLC gradient (c) t_R 25.9 min, isolated amount 1.0 mg; UV (MeCN−H$_2$O) λ_{max} 209, 279, 375, 470 nm; ^{1}H NMR data, see Table 5; ^{13}C NMR data, see Table 6; LCEIMS *m/z* 566 [M]$^+$; HRESIMS *m/z* 567.1607 [M + H]$^+$ (calcd for $C_{28}H_{27}N_2O_{11}$, 567.1615).

Acknowledgment. We thank Sonja Rosenlöcher for technical assistance, and Dr. Daniel J. Fowler for linguistic support in the preparation of the manuscript. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

(1) Cooke, R. G.; J. M. Edwards. *Prog. Chem. Org. Nat. Prod.* **1981**, *40*, 153−190.
(2) Luis, J. G.; Echeverri, F.; Quiñones, W.; Brito, I.; Lopez, M.; Torres, F.; Cardona, G.; Aguiar, Z.; Pelaez, C.; Rojas, M. *J. Org. Chem.* **1993**, *58*, 4306−4308.
(3) Greca, M. D.; Lanzetta, R.; Molinaro, A.; Monaco, P.; Previtera, L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 311−314.
(4) Hölscher, D.; Schneider, B. *J. Nat. Prod.* **2000**, *63*, 1027−1028.
(5) Cooke R. G.; Thomas R. L. *Aust. J. Chem.* **1975**, *28*, 1053−1057.
(6) Hölscher, D.; Schneider, B. *Phytochemistry* **1997**, *45*, 87−91.
(7) Dora, G.; Xie, X.-Q.; Edwards, J. M. *J. Nat. Prod.* **1993**, *56*, 2029−2033.
(8) Cooke, R. G.; Segal, W. *Aust. J. Chem.* **1955**, *8*, 413−421.
(9) Edwards J. M.; Weiss, U. *Phytochemistry* **1974,** *13*, 1597−1602.
(10) Edwards, J. M. *Phytochemistry* **1974**, *13*, 290−291.
(11) Cremona, T. L.; Edwards, J. M. *Lloydia* **1974**, *37*, 112−113.
(12) Hölscher, D.; Schneider, B. *Phytochemistry* **1998**, *49*, 2155−2157.
(13) Hölscher, D.; Schneider, B. *Phytochemistry* **1999**, *50*, 155−161.
(14) Edwards, J. M.; Weiss, U. *Tetrahedron Lett.* **1972**, *17*, 1631−1634.
(15) Bazan, A. C.; Edwards, J. M. *Phytochemistry* **1976**, *15*, 1413−1415.
(16) Cooke, R. G. *Phytochemistry* **1970**, *9*, 1103−1106.
(17) Cooke, R. G.; Dagley, I. J. *Aust. J. Chem.* **1979**, *32*, 1841−1847.
(18) Opitz, S.; Otálvaro, F.; Echeverri, F.; Quiñones, W.; Schneider, B. *Nat. Prod. Lett.* **2002**, *16*, in press.
(19) Pauli, G. F. *J. Nat. Prod.* **2000**, *63*, 834−838.
(20) Hennig, L.; Findeisen, M.; Welzel, P.; Haessner, R. *Magn. Reson. Chem.* **1998**, *36*, 615−620.
(21) Sicker, D.; Schneider, B.; Hennig, L.; Knop, M.; Schulz, M. *Phytochemistry* **2001**, *58*, 812−825.

(22) Olah, G. A.; Ku, A. T.; Olah, J. A. *J. Org. Chem.* **1971**, *36*, 3582−3584.
(23) Dhar, K. L.; Mehta, B. K.; Bokadia, M. M. *Chem. Ind.* **1982**, 862.
(24) Porwal, M.; Sharma, S.; Mehta, B. K. *Ind. J. Chem.* **1988**, *27B*, 281−282.
(25) Sharma, S.; Porwal, M.; Mehta, B. K. *J. Ind. Chem. Soc.* **1988**, *65*, 69−70.
(26) Bick, I. R. C.; Blackman, A. J. *Aust. J. Chem.* **1973**, *26*, 1377−1380.
(27) Hölscher, D.; Schneider, B. *J. Chem. Soc., Chem. Commun.* **1995**, 525−526.
(28) Hölscher, D.; Schneider, B. *Nat. Prod. Lett.* **1995**, *7*, 177−182.
(29) Edwards, J. M.; Weiss, U. *Phytochemistry* **1970**, *9*, 1653−1657.
(30) Dahlgreen, R. M. T. *The Families of the Monocotyledons*; Springer: Berlin, 1985; pp 336−339.
(31) Cooke, R. T.; Thomas, R. L. *Austr. J. Chem.* **1975**, *28*, 1053−1057.
(32) Simpson, M. G. *Ann. Missouri Bot. Garden* **1990**, *77*, 722−784.
(33) Hopper, S. D.; Fay, M. F.; Rosetto, M.; Chase, M. W. *Bot. J. Linn. Soc.* **1999**, *131*, 285−299.

NP020083S