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PHLEGMACINS AND ANHYDROPHLEGMACINQUINONES: DIMERIC HYDROANTHRACENES FROM SEEDLINGS OF *CASSIA TOROSA**

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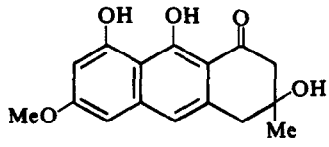
Key Word Index—*Cassia torosa*; Leguminosae; phlegmacin; anhydrophlegmacin-9,10-quinone; hydroanthracene; atropisomer.

Abstract—From seedlings of *Cassia torosa* four dimeric hydroanthracenes have been isolated. Two, a pair of atropisomeric dimers consisting of two molecules of torosachrysone, were identified as phlegmacin A_2 and B_2, the enantiomers of phlegmacin A_1 and B_1 obtained from a higher fungus, *Cortinarius odorifer*. The other pair of atropisomers, based on torosachrysone and physicion, were identified as anhydrophlegmacin-9,10-quinones A_2 and B_2.

INTRODUCTION

Previously we reported the isolation of several anthraquinones and a hydroanthracene, torosachrysone (1), from the seeds of *Cassia torosa* Cavanilles [1]. Recently, germichrysone (2), another hydroanthracene, was isolated as a characteristic constituent of the seedlings of this plant [2]. During these studies four dimeric hydroanthracenes, a pair of atropisomeric dimers consisting of two molecules of torosachrysone (1) and the other pair of atropisomeric dimers consisting of torosachrysone (1) and physcion (3) have been isolated from the seedlings. This paper describes the isolation and the identification of these dimeric compounds, and also discusses their stereochemistry and biogenesis.



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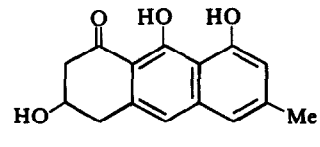
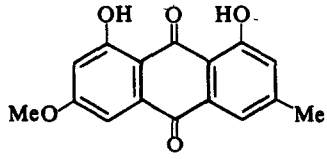
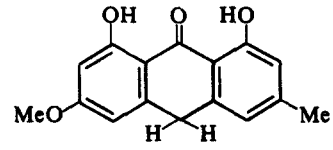


(2) Germichrysone



(3) Physcion



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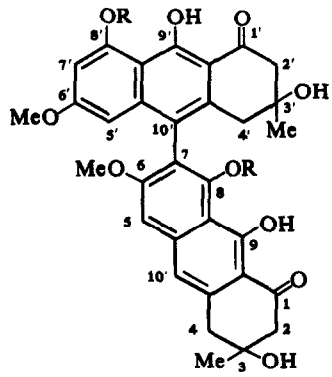
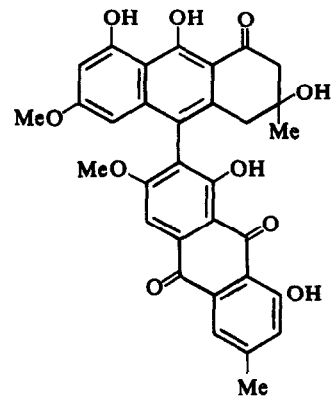


(5) R = H
(6) R = Ac



(7)

* Part 10 in the series 'Studies of the Constituents of Purgative Crude Drugs'. For part 9 see ref. [2].

RESULTS AND DISCUSSION

Dimeric hydroanthracenes, H and I, obtained from polar fractions of column chromatography of C_6H_6 extracts showed different R_f values on TLC and completely opposite ORD curves, whereas their UV, IR and MS were almost identical to each other. In the PMR spectra of compounds H and I, significant differences were observed in the signals due to $-CH_2-$ groups, while the other signals were practically identical. The UV absorption showed the presence of hydroanthracene chromophore [1, 2] and the MS showed molecular ions at *m/e* 574 (M^+; $C_{32}H_{30}O_{10}$), which corresponded to those of the dimers of torosachrysone (**1**). They showed the base peaks at *m/e* 270 corresponding to physcion-anthrone (**4**), which was obtained chemically by the thermal decomposition of the dimers. On treatment with HCl–HOAc, the dimers gave dianhydro-derivatives (**6**), which also yielded physcion-anthrone (**4**) on pyrolysis. The PMR spectra revealed the presence of following functional groups in the dimers; two phenolic, two enolic and two alcoholic OH groups, two Me groups, two OMe groups, four $-CH_2-$ groups, and four aromatic protons, and indicated that their planar structures (**5**) was identical to that of phlegmacin B_1 which had been reported as the constituent of the fungus, *Cortinarius odorifer* Britz. (Agaricales) [3]. The strong Cotton effects in ORD have indicated that H and I are atropisomers caused by restricted rotation around biphenyl linkage and, at the same time, they must be diastereiosomers as indicated by differences in R_f. Attempts to racemize the chiral biphenyl bond resulted in the formation of complex mixtures probably arising from dehydration products. The result indicates that the both H and I are natural products and not artifacts formed during the isolation process by the racemization of one of the isomers.

Direct comparison using TLC and ORD was required to clarify the stereochemical relationship between the three phlegmacins, because their spectral data were almost identical. A sample of phlegmacin obtained from *C. odorifer* gave two spots on TLC, and chromatographic separation afforded two compounds which showed almost identical spectral data except ORD curves as in compounds H and I. The fungal compound showing a negative Cotton effect was defined as phlegmacin B_1 and the other compound showing a positive Cotton effect was named phlegmacin A_1 according to the nomenclature proposed by Steglich [4]. Although an atropisomeric

Table 1. ^{1}H PMR data of dimeric hydroanthracenes obtained from the seedlings of *Cassia torosa*

	2-CH_2	3-Me	3-OH	4-CH_2	10-H	5-H	6-OMe	8-OH	9-OH	
Phlegmacin B_2 (H)	2.84 *br.s*	1.47 *s*	2.05 *s*	3.07 *br.s*	6.93 *s*	6.63 *s*	3.73 *s*	9.90 *s*	15.98 *s*	
					22%	22%				
Phlegmacin A_2 (I)	2.82 *br.s*	1.44 *s*	2.04 *s*	3.08 *br.s*	6.93 *s*	6.65 *s*	3.76 *s*	9.83 *s*	15.98 *s*	
					25%	23%				
	2′-CH_2	3′-Me	3′-OH	4′-CH_2	5′-H	6′-OMe	7′-H	8′-OH	9′-OH	
Phlegmacin B_2 (H)	2.82 *br.s*	1.30 *s*	2.04 *s*	2.72 *d*	6.10 *d*	3.65 *s*	6.43 *d*	10.07 *s*	16.47 *s*	
				2.90 *d*	(*J* = 2.5)		(*J* = 2.5)			
				(*J* = 17)						
Phlegmacin A_2 (I)	2.73 *br.s*	1.28 *s*	2.04 *s*	2.63 *d*	6.13 *d*	3.64 *s*	6.46 *d*	10.08 *s*	16.45 *s*	
				2.85 *d*	(*J* = 2.5)		(*J* = 2.5)			
				(*J* = 17)						
	2-CH_2	3-Me	3-OH	4-CH_2	5-H	6-MeO	7-H	8-OH	9-OH	10-H
Torosachrysone (**1**)	2.80 *br.s*	1.30 *s*	2.00 *s*	2.98 *br.s*	6.60 *d*	3.84 *s*	6.40 *d*	9.87 *s*	15.88 *s*	6.95 *s*
					(*J* = 2.5)		(*J* = 2.5)			
Physcion (**3**)	2-H	3-Me	4-H	5-H	6-OMe	7-H	8-OH	1-OH		
	7.04 *br.s*	2.45 *s*	7.57 *br.s*	7.32 *d*	3.92 *s*	6.60 *d*	12.05 *s*	12.26 *s*		
				(*J* = 2.5)		(*J* = 2.5)				
	2-H	3-Me	4-H	5-H	6-OMe	8-OH	1-OH			
Anhydrophlegmacin-9,10-quinone B_2 (J)	7.05 *br.s*	2.47 *s*	7.59 *br.s*	7.51 *s*	3.64 *s*	11.98 *s*	12.43 *s*			
		31%	32%							
Anhydrophlegmacin-9,10-quinone A_2 (K)	7.08 *br.s*	2.41 *s*	7.58 *br.s*	7.53 *s*	3.70 *s*	11.93 *s*	12.31 *s*			
	2′-H	3′Me	3′-OH	4′-CH_2	5′-H	6′-OMe	7′-H	8′-OH	9′-OH	
Anhydrophlegmacin-9,10-quinone B_2 (J)	2.73 *br.s*	1.34 *s*	1.90 *s*	2.72 *d*	6.08 *d*	3.84 *s*	6.48 *d*	10.28 *s*	16.73 *s*	
				2.87 d	(*J* = 2.5)		(*J* = 2.5)			
				(*J* = 17)		24%	31%			
Anhydrophlegmacin-9,10-quinone A_2 (K)	2.83 *s*	1.31 *s*	–	2.60 *d*	6.07 *d*	3.82 *s*	6.46 *d*	10.14 *s*	16.66 *s*	
				2.84 *d*	(*J* = 2.5)		(*J* = 2.5)			
				(*J* = 17)						

*Spectra were recorded in $CDCl_3$ at 100 MHz. Values are given in ppm (δ) relative to TMS as internal standard. Numbers in parenthesis denote coupling constants in Hz; *s* (singlet), *br.s* (broad singlet), *d* (doublet). Arrows and figures in % indicate enhancement in NOE experiments.

pair of 8′-methylphlegmacins had been isolated from *Corticarius percomis* Fr. [4], it was the first time that phlegmacin A_1 was so far isolated from *C. odorifer*. Direct comparison of phlegmacins A_1 and B_1 with our samples obtained from the seedlings clarified the stereochemical relationships among four phlegmacins. Although compound I showed the same R_f (0.25) as that of phlegmacin B_1, it showed a positive Cotton effect and a completely reverse ORD curve to that of phlegmacin B_1. The same relation was observed between compound H and phlegmacin A_1 (R_f 0.28). Thus it became clear compounds H and I are the enantiomers of phlegmacins B_1 and A_1 and should be named phlegmacin B_2 and phlegmacin A_2 respectively. PMR signals were assigned as shown in the Table by the aid of NOE experiments and also by the shifts of aromatic protons in the corresponding diacetates (6). Acetylation caused significant shifts of aromatic protons. One of the singlets (δ 6.6) and one of the doublets (δ 6.1) were shifted down field by 0.4 and 0.6 ppm, respectively, to be assigned to the aromatic protons *para* to the phenolic OH groups.

Due to the presence of the other chiral centres, the pair of isomers obtained from either *C. torosa* or *C. odorifer* are not only atropisomers but also diastereoisomers. Occurrence of two pairs of phlegmacins from two different sources indicates that the biosynthesis of monomeric moiety, torosachrysone (1), proceeds stereospecifically to produce (+)-torosachrysone in the higher plant and probably (−)-torosachrysone in the fungus, whereas the dimerization reactions in both living systems are not stereospecific.

From less polar chromatographic fractions, another pair of atropisomeric dimers, compounds J (R_f 0.62) and K (R_f 0.59), $C_{32}H_{26}O_{10}$, have been isolated. They showed positive Mg-acetate reaction indicating a quinone nature and almost identical spectral data to each other except ORD, in which the Cotton effects were completely opposite. PMR spectra revealed the presence of three phenolic, one enolic and one alcoholic OH groups, two Me groups, two $-CH_2-$ groups, two MeO groups and five aromatic protons. Comparison of the PMR spectra with those of (1) and (3) as well as the phlegmacins (5) indicated that the quinonoid dimers contain both torosachrysone and physcion moieties. The presence of physcion moiety was verified by thermal decomposition yielding physcion instead of physcion-anthrone yielded in the thermal decomposition of phlegmacins (5). By the comparison of PMR spectra with those of phlegmacins linkages between two monomeric moieties was located between C-10′ of torosachrysone moiety and C-7 of physcion moiety. This planar structure (7) is anhydrophlegmacin-9,10-quinone, the 8′-methyl ether of which has been isolated from *Cortinarius percomis* Fr. [4] along with 8′-methylphlegmacin A_1 and B_1. If we assume that the quinonoid dimers (7) are derived from corresponding phlegmacins (5) by dehydration followed by oxidation, compound J and K should be named anhydrophlegmacin-9,10-quinone B_2 and A_2 respectively according to the signs of their Cotton effects. The PMR signals were assigned as shown in the Table by the comparison with those of phlegmacins (5). The signals due to the torosachysone moieties, especially the signals of $-CH_2-$ groups, are well accord with the corresponding signals of phlegmacins (6).

Recently, dimeric hydroanthracenes possessing similar structures were reported as the toxic constituents of *Karwinskia humboldtiana* Zucc. (Rhamnaceae) [5].

EXPERIMENTAL

Materials were the same as were used in previous report [2]. TLC was carried out with Si gel plate impregnated with *N*/2 oxalic acid developed with C_6H_6–EtOAc (4:1).

Extraction and isolation. C_6H_6 extracts of the seedlings (from 1 kg seeds) were chromatographed over Si gel using C_6H_6–EtOAc (4:1). The eluents were divided into 7 fractions by monitoring with TLC as described in previous paper [2]. Fr. 3 and 4 afforded crude compound J (50 mg) and compound K (80 mg) respectively. Crude compounds H (40 mg) and I (40 mg) were precipitated from the concentrated solution of Fr. 6 and 7 respectively.

Phlegmacin B_2 (Compound H) (5). Crude crystals obtained from Fr. 6 were further purified by Droplet Counter Current Chromatography (DCC) [6] using upper and lower phases of a solvent mixture, C_6H_6–$CHCl_3$–MeOH–H_2O (5:5:7:2). Resinous substances contained in the crude sample were removed by this process and after repeated recrystallization from Me_2CO compound H was obtained as dark brown rods, mp 198–200° (dec). UV $\lambda_{max}^{dioxane}$ nm (log ε): 233 (4.62), 278 (5.01), 320 (4.16), 333 (4.25); IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1630, 1615, 1585; MS 70 eV *m/e* (rel. int.): 574 (M^+; 11.1), 556 (M^+-H_2O; 12.2), 538 (M^+-2H_2O; 84.2), 270 (100); High Resolution MS: Found; 538. 1608, Calcd. for $C_{32}H_{26}O_8$ (M^+-2H_2O); 538.1626; ORD [α] × 10^{-4} (nm): −50.7° (275), 0° (288). +7.5° (295), 0° (360).

Phlegmacin A_2 (Compound I) (5). Powder obtained from Fr. 7 was further purified with DCC using the same solvent system as for compound H. Compound I was finally obtained as dark brown powder, mp 194–5° (dec). UV, IR and MS were almost identical to those of compound H. High Resolution MS: Found; 538.1614; Calcd. for $C_{32}H_{26}O_8$ (M^+-2H_2O); 538.1626; ORD [α] × 10^{-4} (nm): +48.6° (275), 0° (288), −8.1 (295), −0.2 (360).

8,8′-Diacetylphlegmacin B_2 (7). Phlegmacin B_2 was acetylated with Ac_2O and C_5H_5N. After usual work up, the product was chromatographed over Si gel; A yellow fraction eluted with C_6H_6–Me_2CO (2:5) gave yellow needles mp 137°. IR ν_{max}^{KBr} cm^{-1}: 3440, 2940, 1770, 1750, 1620. PMR (100 MHz; $CDCl_3$): 1.23, 1.43 (3H × 2, *s* × 2, Me × 2). 2.20, 2.40 (3H × 2, *s* × 2, Ac × 2), 2.64, 2.84 (1H × 2, *d* × 2, *J* = 17, CH_2), 2.68 (2H, *s*, CH_2), 2.82 (2H, *s*, CH_2), 3.12 (2H, *s*, CH_2), 3.60, 3.80 (3H × 2, *s* × 2, OMe × 2), 6.42, 6.70 (1H × 2, *d* × 2, *J* = 2,5, ArH × 2), 7,02, 7.08 (1H × 2, *s* × 2, ArH × 2), 14.50, 15.00 (1H × 2, *s* × 2, OH × 2).

8,8′-Diacetylphlegmacin A_2 (6). This acetate was obtained as yellow powder mp 178°. IR ν_{max}^{KBr} cm^{-1}: 3340, 2940, 1770, 1750, 1620. PMR (100 MHz; $CDCl_3$): 1.23, 1.43 (3H × 2, *s* × 2, Me × 2), 2.20, 2.40 (3H × 2, *s* × 2, Ac × 2), 2.54, 2.74 (1H × 2, *d* × 2, *J* = 17, CH_2), 2.80 (4H, *s*, CH_2 × 2), 3.10 (2H, *s*, CH_2), 3.65, 3.85 (3H × 2, *s* × 2, OMe × 2), 6.50, 6.72 (1H × 2, *d* × 2, *J* = 2.5, ArH × 2), 7.03, 7.08 (1H × 2, *s* × 2, ArH × 2), 14.56, 15.07 (1H × 2, *s* × 2, OH × 2).

Physcion-anthrone (4) from phlegmacin A_2 and B_2. Phlegmacin B_2 (20 mg) was heated *in vacuo* at 250°. Colourless needles sublimed in a tube showed M^+ 270 in MS and were identified as physcion-anthrone (3) by the comparison of IR with that of authentic sample. Physcion-anthrone was further converted into physcion (3) with KOH–EtOH and identified by mmp. Phlegmacin A_2 (20 mg) also gave physcion-anthrone (5 mg) under the same condition.

Dianhydrophlegmacin A_2 and B_2. Phlegmacin B_2 (20 mg), AcOH (1 ml) and conc HCl (0.01 ml) were heated at 80° for 40 min. Resulted black soln was diluted with H_2O and extracted with EtOAc. The extracts was chromatographed over Si gel and dianhydrophlegmacin B_2 was obtained as colourless needles (5 mg) mp > 270° (from Me_2CO). MS 70 eV *m/e* (rel. int.); 538 (M^+; 85.0), 270 (100), 255 (10.0), 242 (9.0), 227 (9.3). Pyrolysis of dianhydrophlegmacin B_2 (15 mg) afforded physcion-anthrone (4) (4 mg). Phlegmacin A_2 (20 mg) also yielded dianhydrophlegmacin A_2 (5 mg) mp > 270°, which showed identical IR and MS spectra to those of dianhydrophlegmacin B_2.

Anhydrophlegmacin-9,10-quinone B_2 (Compound J). (7). Crude crystals obtained from Fr. 3 were repeatedly recrystallized from

MeOH to give dark red needles, mp 192–3°. UV $\lambda_{max}^{dioxane}$ nm (log ε): 218 (4.63), 268 (4.71), 300 (4.12), 406 (4.16); IR ν_{max}^{KBr} cm^{-1}: 3500, 3400, 2980, 1674, 1640, 1625, 1595; MS 70 eV *m/e*: 570 (M^+), 552 (M^+-H_2O), 284 (base peak). High Resolution MS: Found; 552.1416, Calcd. for $C_{32}H_{24}O_9$(M^+-H_2O); 552.1419. ORD [α] × 10^{-3} (nm): −24.8° (278), 0°, +9.1° (293), 0° (318).

Anhydrophlegmacin-9,10-quinone A_2 (Compound K) (7). Dark red powder obtained from Fr. 4 was repeatedly chromatographed over Si gel to give compound K as dark red powder. UV, IR and MS were identical to those of compound J. ORD [α] × 10^{-3} (nm): +27.1° (275), 0° (288), +9.2° (29.2), 0° (317).

Physcion (3) *from anhydrophlegmacin-9,10-quinone B_2* (7). Pyrolysis of anhydrophlegmacin-9,10-quinone B_2 (10 mg) yielded yellow crystals, which were purified by column chromatography over Si gel to give physcyion (3 mg) mg 205°.

Phlegmacin B_1 and phlegmacin A_1 from Cortinarius odorifer. Crude phlegmacins (300 mg) obtained from *C. odorifer* and generously supplied by Dr. W. Steglich were chromatographed over Si gel with C_6H_6–AcOEt (4:1). First yellow band gave dark brown crude crystals of phlegmacin A_1 (100 mg) and second yellow band gave crude phlegmacin B_1 (120 mg). Phlegmacin A_1 was obtained as dark brown needles mg 195° from Me_2CO. UV $\lambda_{max}^{dioxane}$ nm (log ε): 233 (4.67), 278 (4.93), 320 (4.17), 330 (4.09), 400 (4.33). IR ν_{max}^{KBr} cm^{-1}: 3400, 3930, 1630, 1610, 1590. PMR (60 MHz; $CDCl_3$): 1.28, 1.43 (3H × 2, *s* × 2, Me × 2), 2.82* (6H, *br.s.*, CH_2 × 3), 3.08 (2H, *br.s.*, CH_2), 3.62, 3.73 (3H × 2, *s* × 2, OMe × *s*), 6.11, 1.46 (1H × 2, *d* × 2, *J* = 2.5 Hz, ArH × 2), 6.70, 6.98 (1H × 2, *s* × 2, ArH × 2), 9.98, 10.13, 16.10, 16.60 (1H × 4, *s* × 4, OH × 4). ORD [α] × 10^{-4} (nm): +27.1° (275), °0 (288), −10.9° (294), 1.13° (360). Phlegmacin B_1 was obtained as dark brown needles mp 196° from Me_2CO. UV, IR and MS are identical to those of phlegmacin A_1. PMR (60 MHz; $CDCl_3$): 1.30, 1.46 (3H × 2, *s* × 2, Me × 2), 2.82* (4H, *s*, CH_2 × 2), 2.73, 3.04 (2H × 2, *s* × 2, CH_2 × 2), 3.73, 3.64 (3H × 2, *s* × 2, OMe × 2), 6.14, 6.47 (1H × 2, *d* × 2, *J* = 2.5 Hz, ArH × 2), 6.67, 6.97 (1H × 2, *s* × 2, ArH × 2), 9.94, 10.12, 16.12, 16.59 (1H × 4, *s* × 4, OH × 4). High Resolution MS: Phlegmacin A_1 Found; 538.1624; Phlegmacin B_1 Found; 538.1644, Calcd. for $C_{32}H_{26}O_8$ (M^+-2H_2O); 538.1626.

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* Due to lower resolution these signals appeared as broad singlets.

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