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2,2'-DIMERS OF ALOE-EMODIN AND CHRYSOPHANOL: BIANTHRAQUINONES ISOLATED FROM HUMAN PLASMA AND AROMATIC CASCARA FLUID EXTRACT USP XXI

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Abstract: Compounds 1 and 2 isolated from human plasma were identified as 2,2'-dimers of aloe-emodin and of aloe-emodin and chrysophanol. These compounds apparently originated from consumption of a commercial Cascara preparation which contained these bianthraquinones as their glycosides. Oxydative hydrolysis of the glycosides yielded the aglycones 1, 2 and 3 which were characterized by chemical and electron ionization mass spectrometry and by ^{1}H- and ^{13}C-NMR spectrometry.

Introduction: Plasma of a 36-year old woman showed a red colour. Since it was known that a Cascara containing laxative was regularly taken by this person it was supposed that anthraquinone derivatives could be present in the plasma. Thin layer chromatography of the ether extract of the plasma revealed the presence of two well separated yellow spots which on treatment with a spray reagent for anthraquinones turned to red. However none of the spots corresponded to anthraquinones previously prepared from Cascara bark. It was assumed initially that metabolites were present.
However, when the Cascara extract used in the laxative preparation was examined after oxidative hydrolysis the same spots were detected. Obviously, the substances present in Cascara extract served as precursors for those found in the plasma. Therefore isolation and structure determination of the substances in plasma and extract was undertaken.

Results

Isolation: Compounds 1 and 2 were extracted from acidified plasma and purified by using vacuum liquid chromatography.[1] Their behaviour on TLC, colour reactions and stability towards acidic (HCl) and oxidative hydrolysis (HCl and $FeCl_3$) suggested the presence of anthraquinone aglycones. The compounds were different, however, from the usual anthraquinones isolated from Cascara bark, i.e. chrysophanol, emodin and aloe-emodin. These are formed from the original 8-O-glycosides by acidic hydrolysis, and from the anthrone 8-O,10-C-diglycosides (cascarosides) by oxidative hydrolysis.[2] Compounds 1 and 2 are not detected in Cascara bark. However, oxidative hydrolysis of the extract produced compounds 1 and 2 and a third unknown 3, in addition to the usual anthraquinones. The compounds were purified by column chromatography.

Mass spectrometry: The mass spectral results reported below suggest dimeric structures, which formally originate by loss of two hydrogens from their constituent monomers. Thus, compound 1 [molecular weight (MW) 538] contains six hydroxyl groups and may be derived from aloe-emodin and/or emodin (MW 270). Compound 2 (MW 522) has five hydroxyl groups and may be derived from emodin or aloe-emodin and chrysophanol (MW 254). Likewise, compound 3 (MW 506) is suggested to be a dimer of chrysophanol. The location of the C-C bound connecting the monomeric residues cannot be determined from the mass spectra. However, the lack of monomeric fragment ions shows this linkage to be a very stable one, precluding for instance a 10,10'-location.

The chemical ionization (CI) mass spectrum of compound 1 with isobutane as the reagent gas yields ions at m/z 539 (MH^+), 521 (MH^+-H_2O, m^* 503.6) and 503 (MH^+-2H_2O, m^* 485.6). Less abundant ions observed at m/z 523 and 505 may correspond to loss of water from MH^+ 541 (not observed), indicating partial and possibly thermal reduction of one anthraquinone carbonyl group.

With deuterium oxide as the reagent gas no MD^+ ions are observed. The ion at m/z 521 is shifted to m/z 526, indicating the exchange of at least four protons. With a mixture of isobutane and deuterium oxide as the reagent gas, complete exchange of hydroxyl protons occurs with the neutral deuterium oxide component of the reagent gas, whereas ionization proceeds mainly via protonation by $(CH_3)_3\,C^+$ and to a lesser extent via deuteronation by D_3O^+. Thus, aloe-emodin which contains three hydroxyl protons yielded MH^+ 271 with isobutane CI, MD^+ 275 with deuterium oxide CI and MH^+ 274 (60%) and MD^+ 275 (40%) with a mixture of isobutane and deuterium oxide as the reagent gas. Under the same conditions compound 1 yielded MH^+ 545 and MD^+ 546, suggesting the presence of six hydroxyl groups.

Table 1 gives accurate mass measurements and elemental compositions for the high mass region of the electron ionization (EI) mass spectrum of compound 1. The weak molecular ion at m/z 538 and the associated $[M-H_2O]^{+\cdot}$ and $[M-2H_2O]^{+\cdot}$ ions show the elemental compositions expected for a dimer derived from emodin and/or aloe-emodin. Thermal dehydrogenation and hydrogenation reactions are again observed, as shown by the $[M-2H]^{+\cdot}$ and the $[M+2H-H_2O]^{+\cdot}$ ions.

Table 1. Elemental composition of ions in the EI mass spectrum of compound 1.

Measured mass	Calculated mass	Composition	Relative Abundance	Assignment
538.088	538.090	$C_{30}H_{18}O_{10}$	5	$M^{+\cdot}$
536.071	536.074	$C_{30}H_{16}O_{10}$	7	$[M-2H]^{+\cdot}$
522.089	522.095	$C_{30}H_{18}O_9$	16	$[M+2H-H_2O]^{+\cdot}$
520.074	520.079	$C_{30}H_{16}O_9$	69	$[M-H_2O]^{+\cdot}$
507.065	507.072	$C_{29}H_{15}O_9$	62	$[M+2H-H_2O-CH_3]^+$
505.060	505.056	$C_{29}H_{13}O_9$	72	$[M-H_2O-CH_3]^+$
502.071	502.069	$C_{30}H_{14}O_8$	62	$[M-2H_2O]^{+\cdot}$
491.075	491.077	$C_{29}H_{15}O_8$	100	$[M-H_2O-CO-H]^+$

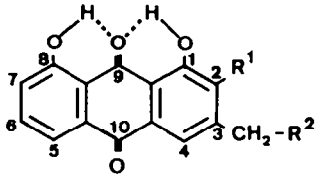
The EI mass spectrum of compound 2 exhibits an abundant molecular ion at m/z 522 and fragment ions corresponding to loss of CH_3 (m/z 507), H_2O (m/z 504), and CH_3 plus H_2O (m/z 489). Thermal reduction occurs to a minor extent only, probably due to the higher volatility of compound 2 as compared to 1. The EI mass spectrum of compound 3 displays

abundant ions $M^{+\cdot}$ and $[M-CH_3]^+$ at m/z 506 and 491, and less intense ions corresponding to loss of water (m/z 488) and to the double charged molecular ion M^{++} (m/z 253).
Silylation of the hydroxyl groups considerably enhanced the sensitivity of the EI mass spectral procedure, permitting identification of compounds 1 and 2 isolated from small quantities of plasma. The characteristic features of these spectra are assembled in Table 2. The spectra show weak molecular ions, very abundant $[M-CH_3]^+$ ions, and relatively abundant doubly charged fragment ions such as $[M-2CH_3]^{2+}$. The silylation gives rise to detection of three products for both 1 and 2, i.e. the completely silylated product, the product with one less trimethylsilyl group and variable amounts of the less abundant product resulting from reduction of one carbonyl group and further silylation. The latter result suggests that reduction may have occurred to some extent prior to silylation.

Table 2. EI mass spectra of trimethylsilyl derivatives of compounds 1 and 2.

Assignment	Complete silylation		Incomplete silylation		Reduced product	
	1 (6TMS)	2 (5TMS)	1 (5TMS)	2 (4TMS)	1 (7TMS)	2 (6TMS)
$M^{+\cdot}$	970	882	898	810	1044	956
$[M-CH_3]^+$	955	867	883	795	1029	941
$[M-2CH_3]^{2+}$	470	426	434	390	507	467.5
$[M-3CH_3]^{2+}$	462.5		426.5		499.5	

NMR spectrometry
A comparison (Table 3) of the ^{1}H-NMR spectra of compounds 1, 2 and 3 with the spectra of aloe-emodin (4) and chrysophanol (5) clearly reveals 2,2'-dimeric structures: 1 and 3 being the symmetric 2,2'-dimers of 4 and 5, respectively, and 2 the mixed 2,2'-dimer.



1, R^1 = 2'-aloe-emodin, R^2 = OH
2, R^1 = 2'-chrysophanol, R^2 = OH
3, R^1 = 2'-chrysophanol, R^2 = H
4, R^1 = H, R^2 = OH (aloe-emodin)
5, R^1 = H, R^2 = H (chrysophanol)

The three-proton pattern of signals observed for protons 5, 6 and 7 on the spectra of 1 and 3 closely reflects that found for 4 and 5, showing the corresponding ring to be unmodified. The spectra of the reference compounds 4 and 5 display proton H-4 as a downfield doublet ($J_{H-4,H-2}$ = 1.5 Hz) and reveal a further coupling of the upfield proton H-2 with either CH_2OH or CH_3. The multiplet signals for H-2 are lacking in the spectra of dimers 1 and 3 and the H-4 protons are detected as downfield singlets. The downfield position of the H-4 protons is analogous to that of the H-5 protons and may be ascribed to the influence of the nearby 10-carbonyl group.

The spectrum of compound 2 displays separate signals for the CH_2OH and CH_3 groups and for the protons H-5, H-6 and H-7 located on either the aloe-emodin or the chrysophanol moiety (Table 3). The 2,2'-linkage is again indicated by the lack of signals corresponding to H-2 and H-2' and by the observation of H-4 and H-4' as downfield singlets.

Table 3. ^{1}H-NMR spectra[a,b] of aloe-emodin (4), chrysophanol (5) and compounds 1, 2 and 3.

Assignment (Multiplicity, J-values)	4	5	1	2	3
CH_3		2.46		2.25	2.25
CH_2OH (q, AB, 13-16 Hz)	4.83,4.86		4.49,4.59	4.47,4.58	
H-2 (m, 1-1.5 Hz)	7.36	7.10			
H-4 (d, 1.5 Hz)	7.81	7.64			
(s)			8.08	8.22,7.87	7.87
H-5 (dd, 7.5 and 1.2 Hz)	7.85	7.81	7.84	7.88,7.90	7.88
H-6 (dd, 7.5 and 8.2 Hz)	7.70	7.66	7.70	7.71,7.73	7.71
H-7 (dd, 8.2 and 1.2 Hz)	7.31	7.28	7.30	7.32,7.33	7.32
1-OH and 8-OH (2 xs)	12.0,12.1	12.0,12.1	11.9,12.4	12.0,12.3	12.1,12.4

a) Spectra are recorded at 250 MHz in $CDCl_3$; δ-values are given in ppm relative to tetramethylsilane as an internal standard

b) Abbreviations : s = singlet, d = doublet, q = quadruplet, m = multiplet, dd = doublet of doublets

The structures of compounds 1 and 2 are confirmed by ^{13}C-NMR spectrometry (Table 4). The assignment of C-atoms in the spectra of aloe-emodin and chrysophanol is based on a comparison (Table 4) with the spectrum of 1,8-dihydroxyanthraquinone.[3] The substituent effects observed for the 3-CH_2OH and 3-CH_3 groups on the chemical shifts of nearby C-atoms 1 to 4 are in agreement with reported values.[4] The spectra of 4 and 5 are used in turn for the assignment of C-atoms in the spectra of dimers 1 and 2. The 2,2'-linkages are revealed by a large upfield chemical shift for C-2 (ca. 6 ppm) and smaller upfield shifts for C-1 (3 ppm), C-3 and C-4. The asymmetric nature of dimer 2 is demonstrated by detection of separate signals for CH_3 and CH_2OH and for several C-atoms belonging to either the aloe-emodin or the chrysophanol moiety.

Table 4. ^{1}H-decoupled ^{13}C-NMR spectra[a] of 1,8-dihydroxyanthraquinone[b] (6), aloe-emodin (4), chrysophanol (5) and compounds 1 and 2.

Assignment of C-atom[c,d]	6	4	5	1	2[d]
1/8	161.1	163.0 and 162.5	162.7 and 162.4	159.6 (1)	159.7 (1/1')
				162.7 (8)	162.7 (8/8')
2/7	124	121.5 (2)	124.5 and 124.3	128.1 (2)	128.4 (2)
		124.5 (7)			130.1 (2')
				124.9 (7)	124.8 (7/7')
3/6	137.1	152.4 (3)	149.2 (3)	149.6 (3)	149.6 (3)
					148.7 (3')
		137.0 (6)	136.8 (6)	137.6 (6)	137.8 and 137.4 (6/6')
4/5	119.2	117.9 (4)	121.8 (4)	120.3 and 120.0	118.9 (4)
					121.9 (4')
		119.9 (5)	119.8 (5)		120.7 and 120.2 (5/5')
9	192.5	e	192.5	192.8	192.9 and 192.7 (9/9')
10	180.9	e	181.8	181.3	181.6 and 181.4 (10/10')
11/14	133.1	133.7	133.6 and 133.3	133.9 and 133.6	133.8,133.7,133.3 and 133.2 (11/14 and 11'/14')
12/13	115.8	115.9	115.9 (12) 113.7 (13)	115.9 and 115.2	115.9 and 115.3 (12/13 and 12'/13')
CH_2OH		63.8		63.0	62.8
CH_3			22.2		20.2

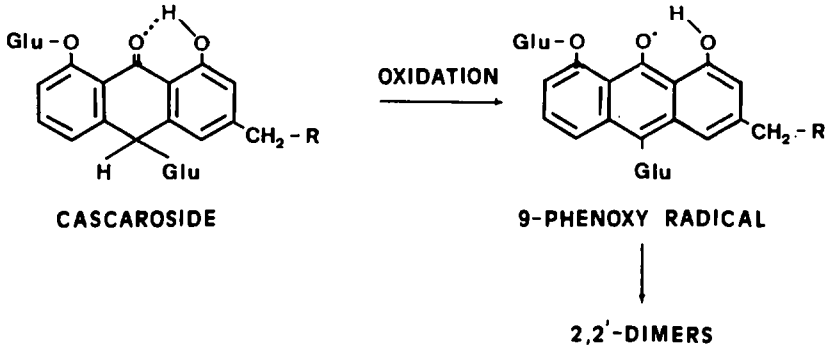
[a] Spectra were recorded at 62.9 MHz in $CDCl_3$ solution; δ-values are given in ppm relative to tetramethylsilane as internal standard. [b] Spectrum taken from reference 3. [c] Where possible C-positions are distinguished further as mentioned in parentheses, e.g. positions 2 and 7 in the spectrum of 4. [d] Aloe-emodin and chrysophanol positions of dimer 2 are indicated by non-primed and primed figures, respectively. [e] Signal not detected during to low solubility of 4 in $CDCl_3$.

Discussion

2,2'-Bianthraquinones have been isolated previously from the bark of Cassia siamea. They were called cassiamin A, B, and C and were identified as 2,2'-dimers of emodin and chrysophanol (A), emodin (B), and chrysophanol (C).[5] Probably compound 3 is identical with cassiamin C. However, the spectral data cannot be compared directly, as the NMR and mass spectral methods at the time of the previous investigation were not sufficiently sensitive for examining the underivatized compound and the authors had to resort to the more volatile and more soluble tetraacetate and tetramethylether derivatives.

A related 7,7'-dimer of chrysophanol designated as chrysotalunin has been isolated from soil by McGrath.[6] Marked differences can be noticed between the published ^{1}H-NMR data of the acetates of chrysotalunin and cassiamin C.[5,6] Furthermore, the EI mass spectrum of compound 3 is dominated by the loss of a sterically crowded 3-methyl group, while this fragmentation pathway is not mentioned for the 7,7'-dimer.

The origin of compounds 1, 2 and 3 in the cascara extract used for preparation of the laxative remains uncertain. We found that 2,2'-dimerisation of cascarosides, i.e. the anthrone 8-O-10-C-diglucosides can be effected by heating at the air under slightly alkaline conditions. This reaction may be compared to the oxidative coupling of anthrones at the 10,10'-positions. Oxidation of a cascaroside likewise will remove the labile 10-H-atom to afford a 9-phenoxy radical which displays radical character also at carbon atoms 10, 2 and 7, and 4 and 5. Steric hindrance imparted by the glucose moieties will prevent dimerisation, however, except at the more accessible 2-position. Furthermore, the reactivity at position 2 may be increased by sharing of the 1-OH hydrogen by the 9-O radical center.



Experimental

Materials: Aromatic fluid extract USP XXI was obtained from Penick Corporation (Lyndhurst, N.J.). Lyophilized plasma was obtained from Prof. S. Bottomley (Medical Center, Veterans Administration, Oklahoma City). All chemicals were of reagent grade.

Apparatus: ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker Cryospec WM-250 instrument (250 MHz for ^{1}H, 62.9 MHz for ^{13}C). Chemical shifts are given in parts per million relative to tetramethylsilane as an internal standard.

IR spectra were recorded on a Perkin Elmer 197.

Electron ionisation mass spectra were obtained on a Kratos AEI MS-902S instrument: ionizing energy 70 eV, accelerating voltage 8 kV and direct insertion into the ion source operated at 200-250°C as required. Accurate mass measurements were performed at a dynamic resolution of 7,500 using a VG Analytical 2010 Data System.

Chemical ionisation mass spectra were recorded on a Kratos AEI MS12 instrument modified for chemical ionisation.

Trimethylsilyl derivatives of the anthraquinone dimers were prepared by reaction of the compounds (10-100 µg) dissolved in pyridine (0.05 ml) with N,O-bis (trimethylsilyl) trifluoroacetamide (0.03 ml) at room temperature for 30 minutes.

Chromatography: Plasma (1 to 2 ml) or extract (50 to 100 mg) are heated during 4 hours in a boiling water bath with 20 ml water, 12 ml conc. hydrochloric acid and 2 g ferric chloride. After cooling, extraction with ether, and evaporation of the solvent the residue is dissolved in 5 ml ethanol and 10 ml N potassium hydroxide. The mixture is heated for 10 min. in a boiling water bath, cooled and extracted with 10 ml petroleum ether and 10 ml ether. After adding 2 ml of conc. hydrochloric acid to the water phase the free compounds are extracted with ether. The residue obtained after evaporation is dissolved in 1 ml of a mixture of chloroform-methanol (1:1). This solution is used for chromatography on thin layer plates of silica gel (Merck, Darmstadt).

Solvent system A :	ether, chloroform, formic acid (50:50:1.5)
Solvent system B :	hexane, benzene, acetic acid (60:40:20)
Spray reagent :	freshly prepared solution of 5 g potassium hydroxide in 70 ml of ethanol 50% and 30 ml formamide. Heating for 15 min. at 110°C.
Reference solution :	aloe-emodin, emodin, chrysophanol (1 mg/ml)
Rf (System A, System B) :	1, 0.16, 0.1; 2, 0.56, 0.28; 3, 0.72, 0.59; aloe-emodin : 0.48, 0.23; emodin, 0.67, 0.3; chrysophanol, 0.72, 0.65.

Isolation from aromatic Cascara fluid extract: About 50 g aromatic Cascara fluid extract USP XXI are heated in a boiling water bath for 4 hours with 1000 ml of water, 540 ml of conc. hydrochloric acid and 90 g ferric chloride.
After cooling anthraquinone aglycones are extracted with 2 x 500 ml of ether. The combined ether extracts are washed with 3 x 200 ml of water, dried over anhydrous sodium sulphate, and evaporated on 5 g Celite. The residue is chromatographed over silica gel 0.063-2 mm (70 g) by elution with a mixture of chloroform, ether and formic acid (100:100:0.25).
The first fractions contain compound 3, chrysophanol and physcione. Further eluates contain compound 2, emodin and aloe-emodin. Finally the last fractions contain only compound 1. These fractions were evaporated and the residue was dissolved in 2 ml dichloromethane and poured into 20 ml hexane. The yellow precipitate afforded pure 1 (8 mg). This isolation procedure was repeated several times in order to obtain 65 mg of 1. UV (nm)(MeOH): 229, 260, 289(sh), 434. IR (cm^{-1})(KBr): 3400, 1669, 1619. Compound 2 was isolated by chromatography of the combined fractions over silica-gel 0.040-0.063 mm (70 g) by elution with a mixture of dichloromethane, hexane, ether and formic acid (320:40:40:0.5). Fractions containing 2 were evaporated. The residue was dissolved in 2 ml dichloromethane and poured into 20 ml hexane. The yellow precipitate (5 mg) was pure 2. The isolation procedure was repeated several times in order to obtain 42 mg of 2. UV (nm)(MeOH): 229, 260, 289(sh), 434. IR (cm^{-1})(KBr): 3400, 1670, 1619. Compound 3 was isolated by chromatography of the combined fractions over silica-gel 0.040-0.063 mm (60 g) by elution with a mixture of dichloromethane, hexane and formic acid (350:100:1). Fractions containing 3 were evaporated. The residue was dissolved in 2 ml dichloromethane and poured into 20 ml hexane. The yellow precipitate (6 mg) was pure 3.

Isolation from plasma: About 8 g of lyophilized plasma was heated for 45 min. in a boiling water bath with 250 ml water and 750 ml conc. hydrochloric acid. After cooling 1000 ml water were added and the mixture was extracted twice with 500 ml ether. After evaporation of the ether on 5 g celite the compounds were separated by vacuum liquid chromatography[1] on silica-gel 0.015-0.040 mm with a mixture of chloroform, tert-butylmethyl ether and formic acid (90:10:0.3). Fractions containing compound 1 or 2 were combined and further treated as described, yielding 3 mg of compound 1 and 2 mg of compound 2.

References

1. N.M. Targett, J.P. Kilcoyne and B. Green, J. Org. Chem. 44, 4962 (1979)
2. J.N. Fairbairn and S. Simic, J. Pharm. Pharmacol. 15, 325 (1963)
3. Y. Berger and A. Castonguay, Org. Magn. Reson. 11, 375 (1978)
4. D.F. Ewing, Org. Magn. Reson. 12, 499 (1979)
5. V.B. Patil, A.V. Rama Rao and K. Venkataraman, Indian J. Chem. 8, 109 (1970)
6. D. McGrath, Chem. and Ind. 1353 (1970)