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ELUCIDATION OF THE SUBSTITUTION PATTERN OF 9,10-ANTHRAQUINONES THROUGH THE CHEMICAL SHIFTS OF *PERI*-HYDROXYL PROTONS

JAN SCHRIPSEMA and DENISE DAGNINO

Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

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Key Word Index—Anthraquinones; ^{1}H NMR; substitution pattern; hydroxyl chemical shift; revision.

Abstract—In 9,10-anthraquinones the chemical shift of a *peri*-hydroxyl proton is affected by the substituents in the other benzenoid ring. These effects are additive. They are useful for the determination of substitution patterns and have been used to revise the structures of six previously reported anthraquinones containing methoxyl, hydroxyl, methylenedioxy and β-methyl substituents. Because the chemical shifts of the other protons are hardly affected by substitutions in the other ring, the characteristic chemical shifts for a wide variety of substitution patterns could be derived.

INTRODUCTION

Anthraquinones are common secondary metabolites. They occur widely in the subclass Asteridae, comprising among others the plant families Rubiaceae, Gesneriacea and Scrophulariaceae. In these plants they are considered to be biosynthetically derived from shikimic acid and mevalonate. Anthraquinones not from the subclass Asteridae, e.g. those which occur in the Rhamnaceae, Polygonaceae, Leguminosae and in fungi and lichen, are polyketides. Several hundred different anthraquinones are known, differing in the nature and positions of the substituents. For the structural elucidation of unknown anthraquinones the nature of the substituents, the substitution pattern of both rings, and the orientation of one ring relative to the other need to be determined.

In this paper, the determination of the relative position of substituents in one ring through the ^{1}H NMR chemical shifts of *peri*-hydroxyl protons in the other benzenoid ring has been investigated.

RESULTS AND DISCUSSION

peri-Hydroxyl protons display sharp singlets in the ^{1}H NMR spectrum recorded in $CDCl_3$, due to strong hydrogen bonding to the adjacent carbonyl. These signals usually appear between 12 and 14 ppm. Little attention has been given to the exact value of these chemical shifts and often they have not even been the hydroxyl is located. Moreover, an upfield shift of these signals would be expected if the carbonyl has additional hydrogen bonding to another hydroxyl as in 1,8-dihydroxy substitution. The chemical shifts of these signals might thus be a useful tool for the structural elucidation of unknown anthraquinones.

To determine the effects of substituents in the other ring on the chemical shifts of the *peri*-hydroxyl protons, the ^{1}H NMR data for anthraquinones, collected from the literature, were investigated. Only anthraquinones with methoxyl, hydroxyl, methylenedioxy and β-methyl substituents were taken into consideration in this study.

From the collected data it became obvious that the chemical shifts of the protons in one ring, except for those of *peri*-hydroxyl protons, hardly changed (rarely more than 0.1 ppm) when the substitution pattern of the other ring was changed. Only one significant effect could be noticed: a methoxyl (instead of a proton or hydroxyl) in position 5 relative to an aromatic proton at position 1 would give an upfield shift of about 0.1 ppm of the signal of the aromatic proton.

A suitable set of data for the calculation of the substituent effects on *peri*-hydroxyl protons was found in the publication of Simoneau and Brassard [1], in which a series of 2-methylquinizarins was synthesized and the complete ^{1}H NMR data were reported. Based on these data the substituent effects, listed in Table 1, were calculated. These substituent effects were subsequently confirmed by testing them on data obtained

tions were obtained, these being the values for a system with no substitution in the other ring. These typical values are listed in Table 2.

By using the data in Tables 1 and 2, the ^1H chemical shifts of nearly all 9,10-anthraquinones with hydroxyl, methoxyl, methylenedioxy and β-methyl substituents can be predicted within 0.1 ppm. In Fig. 1 this is illustrated for two isomeric structures. The accuracy of the predicted values is usually better than 0.1 ppm. In a few cases a larger difference was noted between the calculated and the reported values for *peri*-hydroxyl protons. In all these cases the reported values were up to 0.3 ppm lower than the calculated value. This might have been due to a significant amount of water in the measurement solution.

In a number of cases inconsistencies became apparent between published data and the proposed structures and they will be discussed below.

The chemical synthesis of anthraquinones, especially by the group of Brassard, had already shown that in many cases reported structures for anthraquinones were incorrect [1–7], and based on their studies the structures of a number of anthraquinones has already been revised.

Revised structures

Comparison of the chemical shifts reported in the literature with those calculated for the reported structures using the data in Tables 1 and 2 revealed that in a number of cases anthraquinones had been wrongly identified.

The structures of three anthraquinones reported by Wijnsma *et al.* [8] from callus cultures of *Cinchona ledgeriana* were revised by Simoneau and Brassard [3]. Purpurin 1-methyl ether (2,4-dihydroxy-1-methoxyanthraquinone, **1**) was revised as 1,3-dihydroxy-2-methoxyanthraquinone (**3**). The compound originally reported as 1,3-dihydroxy-2,5-dimethoxyanthraquinone (**4**) was revised as 2,5-dihydroxy-1,3-dimethoxyanthraquinone (**7**). Finally, the compound which was reported as 4-methoxy-1,3,5-trihydroxyanthraquinone (**2**) was considered to be 1,3,5-trihydroxy-2-methoxyanthraquinone (**5**). The first two revisions are supported by the results presented here, but the third is only partly correct. The compound has two hydroxyl signals in the proton spectrum at δ 12.08 and 12.49, while for the synthesized product values of δ 12.63 and 13.18 were reported [3]. This difference was ignored by Simoneau and Brassard [3], but as illustrated in Fig. 1 it clearly indicates a 1,8-dihydroxy substitution pattern for the compound isolated from *Cinchona*. For a 1,5-dihydroxy substitution (as proposed by Simoneau and Brassard [3]) the calculated shifts for the *peri*-hydroxyl protons are δ 12.86 and 13.27, while for a 1,8-dihydroxy substitution the calculated shifts are δ 12.09 and 12.50. It is thus clear that the compound isolated from *Cinchona* is 1,3,8-trihydroxy-2-methoxyanthraquinone (**6**).

Table 1. Substituent effects on the ^1H NMR chemical shift of a *peri*-hydroxyl proton in 9,10-anthraquinones (calculated from ref. [1]). The *peri*-hydroxyl for which the calculation is performed is considered to be located at C-1

5-OH	+0.13
5-OCH_3	−0.02
6-OCH_3	+0.13
7-OCH_3	−0.09
8-OH	−0.64
8-OCH_3	+0.35

At the same time as Simoneau and Brassard [3] published their revision of the above structures, two publications appeared in which purpurin 1-methyl ether (**1**) was reported as a constituent of callus cultures of *Cinchona pubescens* [9] and of cell suspension cultures of *C. ledgeriana* [10], respectively, with reference to the original incorrect data [8]. Indeed, even seven years later this compound was reported following reference to the wrong data, as a constituent of *Galium spurium* [11]. In all these cases compound **3** was isolated.

In the publication of González *et al.* [12] concerning the anthraquinones from *Cassia greggii*, the reported data for all of the seven new anthraquinones do not fit with the derived structures. In a recent reinvestigation of this species (under the synonym *Chamaecrista greggii*) by Barba *et al.* [13], the structures of all the seven previously reported anthraquinones were revised. Additionally, nine other anthraquinones were reported in this publication. Barba *et al.* [13] relied in their structure determinations mainly on NOE difference spectrometry and on the solvent shift method. In the latter method the shifts of the methoxyl signals upon change of the solvent from $CDCl_3$ to C_6D_6 are used to discriminate between aromatic methoxyl groups *ortho* to aromatic protons and other methoxyls by the large shifts of the former. Some of the structure assignments of Barba *et al.* [13] are not supported by our present investigation.

Compound 12 of González *et al.* [12], reported as 4,5-dihydroxy-1,6,7-trimethoxy-2-methylanthraquinone (**8**), was revised by Barba *et al.* [13] as 1,5-dihydroxy-4,6,7-trimethoxy-2-methylanthraquinone (**9**). Both structures are not in accordance with the reported UV spectrum [12], with a maximum at 476 nm. This indicates a 1,4-dihydroxy substitution. The signal of an aromatic proton at δ 7.75 is also not in accordance with the 5-hydroxy-6,7-dimethoxy substitution of the A-ring as proposed both by González *et al.* [12] and Barba *et al.* [13]. For such a proton δ 7.41 would be expected (Table 2). Thus, the compound should be revised as 1,4-dihydroxy-5,6,7-trimethoxy-2-methylanthraquinone (**10**). This structure is also supported by the chemical shifts of the *peri*-hydroxyl protons (δ 13.38 and 13.30). Values of δ 13.37 (OH-1) and 13.35 (OH-4) are

Table 2. Typical chemical shifts for different substitution patterns. These values are for a 9,10-anthraquinone unsubstituted in the other ring. Upon substitutions in the other ring, only the values of *peri*-hydroxyl protons will change, as indicated in Table 1. For each substitution pattern the chemical shifts of the substituents and protons at positions 1–4 are given. Within each group the patterns are sorted according to the highest chemical shift of an aromatic proton. In the column labelled 'No.' the number of anthraquinones which were available to obtain the values is given. In the last column the references in which these compounds can be found are given

Substitution pattern	1	2	3	4	No.	References
1,2,3-Substituted:						
2-Hydroxy-1-methoxy-3-methyl	4.02	6.75	2.39	7.92	3	25
2-Hydroxy-1,3-dimethoxy	4.09*		4.01*	7.70	7	8,11,13,26,27
1,2,3-Trimethoxy	4.04*	4.01*	4.00*	7.67	18	11,12,13,14,19,28,29,30
1,2-Dihydroxy-3-methyl	12.82	6.31	2.38	7.68	5	14,29
1,3-Dimethoxy-2-methyl	3.91	2.25	4.02	7.60	3	6
3-Hydroxy-1,2-dimethoxy	4.00*	3.97*		7.60	2	8,13
1,2-Methylenedioxy-3-methoxy	6.33		4.07	7.59	2	11,13
1,2-Dihydroxy-3-methoxy	12.67	5.89	4.07	7.50	1	14
1,3-Dihydroxy-2-methoxy	13.14	4.15		7.47	8	3,11,14,24,27
1-Hydroxy-3-methoxy-2-methyl	12.98	2.19	4.01	7.40	4	6
1-Hydroxy-2,3-dimethoxy	12.80	4.03	4.01	7.41	9	13,14,27,29
1,3-Dihydroxy-2-methyl	13.19	2.23		7.27	1	26
1,2,4-Substituted:						
1,4-Dihydroxy-2-methyl	13.35	2.36	7.14	12.96	12	1,8,24,31
1,4-Dimethoxy-2-methyl	3.90	2.40	7.14	3.98	6	12,30
4-Hydroxy-1-methoxy-2-methyl	3.86	2.36	7.13	13.03	4	13
2-Hydroxy-1,4-dimethoxy	4.00*	6.80	6.97	3.97*	3	3,32
2,4-Dihydroxy-1-methoxy	3.98	6.86	6.86	13.52	3	3,32
1-Hydroxy-2,4-dimethoxy	13.50	3.99	6.79	3.99	1	32
1,2,4-Trimethoxy	3.93*	4.02*	6.79	3.98*	7	3,29,33,34
4-Hydroxy-1,2-dimethoxy	3.96*	3.92*	6.72	13.62	2	3,32
1,4-Dihydroxy-2-methoxy	13.45	3.98	6.70	13.55	4	32,34,35,36
1,2-Substituted:						
2-Hydroxy-1-methoxy	4.03		7.36	8.16	2	8,26
1,2-Dimethoxy	4.03*	4.00*	7.24	8.14	3	10,19,24
1-Methoxy-2-methyl	3.93	2.43	7.61	8.04	6	11,19,28
1,2-Methylenedioxy	6.34		7.16	7.99	4	24,37,38
1-Hydroxy-2-methoxy	12.98	4.03	7.20	7.89	3	24,39,40
1,2-Dihydroxy	12.89	6.24	7.26	7.84	1	4
1-Hydroxy-2-methyl	12.87	2.36	7.51	7.71	15	3,4,8,11,19,24,27
1,3-Substituted:						
1-Methoxy-3-methyl	4.04	7.13	2.48	7.77	14	19,28,29,30,33,34
1-Hydroxy-3-methyl	12.59	7.08	2.45	7.60	21	1,3,6,19,24,25,29,36,41
1,3-Dimethoxy	4.04*	6.80	4.00*	7.48	11	1,19,24,30,33,41
1-Hydroxy-3-methoxy	12.92	6.69	3.94	7.36	11	1,4,19,24,31,42
1,4-Substituted:						
1-Hydroxy-4-methoxy	12.98	7.35	7.40	4.04	2	25
1,4-Dimethoxy	3.99	7.33	7.33	3.99	4	25,30
1,4-Dihydroxy	12.89	7.26	7.26	12.89	7	1,6,19,24,36,41
2,3-Substituted:						
2,3-Dimethoxy	7.70	4.00	4.00	7.70	1	43
2,3-Methylenedioxy	7.68		6.17	7.68	1	37
1-Substituted:						
1-Methoxy	4.06	7.33	7.72	8.00	14	1,3,4,6,10,19,24,30,38
1-Hydroxy	12.69	7.29	7.66	7.83	20	1,3,4,19,25,27,38,39,42
2-Substituted:						
2-Methyl	8.04	2.52	7.55	8.20	14	3,4,10,13,24,27,35,40,43

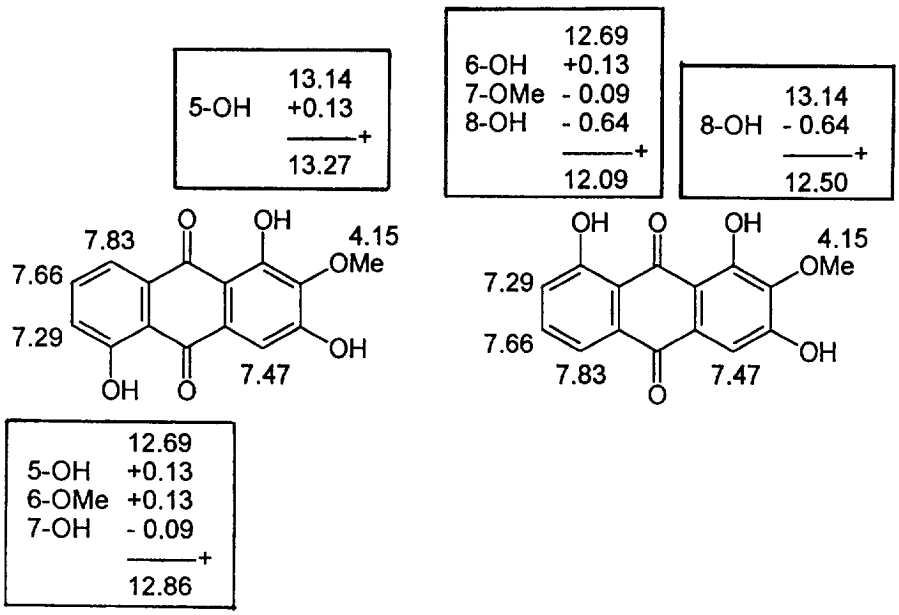
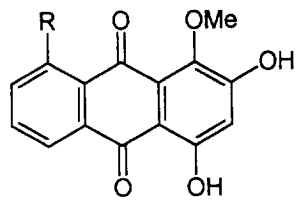
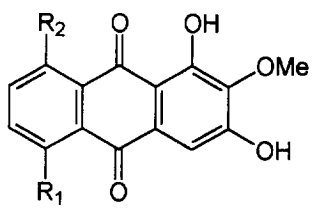


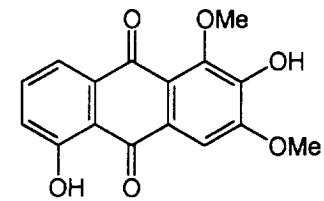
Fig. 1. Calculation example: the chemical shifts of the two isomeric anthraquinones (**5** and **6**) are predicted using the values given in Tables 1 and 2. In this example, both compounds have a 1-hydroxy substituted ring and a 1,3-dihydroxy-2-methoxy substituted ring. The typical chemical shifts of these substitution patterns can be found in Table 2. The chemical shifts of the *peri*-hydroxyl protons in Table 2 are those of 9,10-anthraquinones unsubstituted in the other ring and thus in this example the factors from Table 1 need to be applied as indicated in the boxes to calculate the chemical shifts in the presented structures.



	R
1	H
2	OH



	R_1	R_2
3	H	H



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It has to be noted that the NMR data of this compound are identical to those of 'compound 6' of González *et al.* [12] with a 0.07 ppm shift (except for the extraordinary chemical shift of the methyl of δ 2.82, which the authors did not comment upon, and which probably is a typing error, the true value being 2.28 ppm). 'Compound 6' was obtained from 'compound 5' by treatment with diazomethane (see below).

'Compound 5' of González *et al.* [12], reported as 1,5,7-trihydroxy-4,6-dimethoxy-2-methylanthraquinone (**11**), was revised by Barba *et al.* [13] as 1,4,7-trihydroxy-5,6-dimethoxy-2-methylanthraquinone (**12**). However, the reported value of δ 7.77 for H-8 is too high for a 7-hydroxy-5,6-dimethoxy substituted A-ring; δ 7.60 would be expected (Table 2). A 6-hydroxy-5,7-dimethoxy substitution would be much more probable. Also, the large difference in the chemical shifts of both

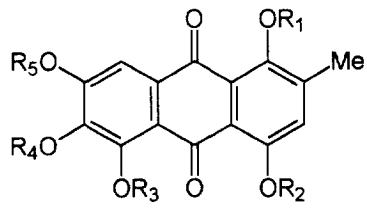
quinone (**13**). Consequently, 'compound 6' reported as **14** by González *et al.* [12], which was obtained from 'compound 5' by methylation with diazomethane, should be 1,4-dihydroxy-5,6,7-trimethoxy-2-methylanthraquinone (**10**), which is also compatible with the data reported by González *et al.* [12] and fits with 'compound 6' being identical to 'compound 12', as was proposed above.

The compound which Barba *et al.* [13] identified as 4,7-dihydroxy-1,5,6-trimethoxy-2-methylanthraquinone (**15**) needs for the same reasons as discussed above for 'compound 5' to be revised as 4,6-dihydroxy-1,5,7-trimethoxy-2-methylanthraquinone (**16**). Additional evidence for this revision is given by the solvent shift method. Upon change of the solvent from $CDCl_3$ to C_6D_6 one of the methoxyl signals displays a 0.68 ppm upfield shift [13], thus classifying it as a methoxyl *ortho* to an aromatic proton. Only in structure **16** is such a methoxyl present. The other revisions made by Barba *et al.* [13] are supported by our present results.

'Compound 1' of González *et al.* [12], reported as 5-hydroxy-1,4,6,7-tetramethoxy-2-methylanthraquinone (**17**), was revised by Barba *et al.* [13] as 4-hydroxy-1,5,6,7-tetramethoxy-2-methylanthraquinone (**18**). This is in accordance with the two singlets from protons on the anthraquinone nucleus at δ 7.13 and 7.66 (Table 2). Furthermore, it is supported by the singlet of a *peri*-hydroxyl proton at δ 13.38. For the revised structure, a chemical shift of δ 13.42 can be calculated in contrast to δ 13.13 which can be calculated for the originally proposed structure. The only inconsistency with this structure would be the NOE effect between the methoxyl signal at δ 4.04 and the signal at δ 7.13, reported by González *et al.* [12], but which could not be reproduced by Barba *et al.* [13]. Another proof in favour of the revised structure **18** is the acylation shift, which was incorrectly used by González *et al.* [12] to support their structure. Upon acetylation of 'compound 1' the proton at δ 7.66 shifted 0.13 ppm upfield. Kitanaka and Takido [14] found that, upon acylation of *peri*-hydroxyls, aromatic protons in *ortho* and *para* positions underwent significant downfield shifts of about 0.3 ppm. From their data, one can also derive that a proton in the 5 position on the other ring would experience a small upfield shift of about 0.1 ppm, as was observed for compound **1** and thus supports the revised structure **18**.

'Compound 8' of González *et al.* [12], originally reported as 5,6-dihydroxy-1,4,7-trimethoxy-2-methylanthraquinone (**19**), was revised as 4,6-dihydroxy-1,5,7-trimethoxy-2-methylanthraquinone (**20**) by Barba *et al.* [13]. The reported chemical shift of the *peri*-hydroxyl proton was δ 13.33, but calculation yielded a value of δ 13.00 for the originally reported structure, while for the revised structure δ 13.42 can be calculated. Methylation with diazomethane yielded 'compound 1' (**18**, after revision).

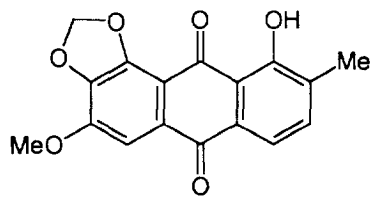
'Compound 9' of González *et al.* [12], reported as

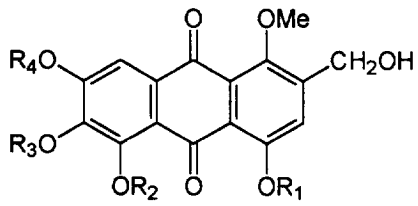


	R_1	R_2	R_3	R_4	R_5
8	Me	H	H	Me	Me
9	H	Me	H	Me	Me
10	H	H	Me	Me	Me
11	H	Me	H	Me	H
12	H	H	Me	Me	H
13	H	H	Me	H	Me
14	H	Me	H	Me	Me
15	Me	H	Me	Me	H
16	Me	H	Me	H	Me
17	Me	Me	H	Me	Me
18	Me	H	Me	Me	Me
19	Me	Me	H	H	Me
20	Me	H	Me	H	Me
21	H	Me	$-CH_2-$		Me
22	Me	H	$-CH_2-$		Me

quinone (**22**) by Barba *et al.* [13]. This revision is in accordance with the reported shifts for the methoxyl groups. The chemical shift δ 4.06 corresponds exactly with the methoxyl in 8-hydroxy-3-methoxy-7-methyl-1,2-methylenedioxyanthraquinone (**23**), isolated from *G. spurium*, and reported by Koyama *et al.* [11]. The low value of the other methoxyl chemical shift (δ 3.86) indicated the 1-methoxy-2-methyl moiety.

'Compound 10' of González *et al.* [12] was reported as 5,7-dihydroxy-1,4,6-trimethoxy-2-hydroxymethylanthraquinone (**24**) and was revised as 4,6-dihydroxy-1,5,7-trimethoxy-2-hydroxymethylanthraquinone (**25**). The A-ring substitution was indicated by the chemical shift of H-8 (δ 7.67) as 5,7-dimethoxy-6-hydroxy. On the basis of the low chemical shift of one methoxyl (δ 3.90) and mass spectral evidence (loss of methane





	R_1	R_2	R_3	R_4
24	Me	H	Me	H
25	H	Me	H	Me

from the molecular ion), the methoxyl in the C-ring had to be at position 1. The structure is also supported by the chemical shift of the *peri*-hydroxyl proton, δ 13.39. For this structure the calculated value is δ 13.42.

'Compound 14' of González *et al.* [12], reported as 5,6-dihydroxy-4,7-dimethoxy-2-methylanthraquinone (**26**), was revised as 4,6-dihydroxy-5,7-dihydroxy-2-methylanthraquinone (**27**). A 4-hydroxy-2-methyl substitution for ring C instead of 4-methoxy-2-methyl was indicated by the signals of aromatic protons at δ 7.07 and 7.58 (Table 2). The signal of the other aromatic proton at δ 7.69 (not δ 7.60 as reported by Barba *et al.* [13]), indicates a 6-hydroxy-5,7-dimethoxy substitution for ring A. The chemical shift of the *peri*-hydroxyl proton, δ 12.90 [12] or 12.97 [13], is in accordance with the value calculated for the revised structure (δ 12.98).

Kazmi *et al.* [15] reported 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**28**) as a new compound. However, the data for this compound are similar to those for przewalskinone B, reported two years before in the same journal by Lu *et al.* [16]. Przewalskinone B was originally reported with the same structure as was reported by Kazmi *et al.* [15], but shortly after the publication of Lu *et al.* [16] the structure of przewalskinone B was revised by Kelly *et al.* [17] as physcion (4,5-dihydroxy-7-methoxy-2-methylanthraquinone, **29**).

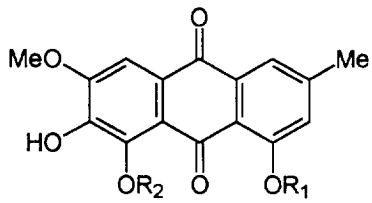
The data for 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**28**) are indeed similar to those for physcion (**29**), but some clear differences can be noted: the chemical shifts of the *peri*-hydroxyl protons and the chemical shifts of the carbonyl carbons in the ^{13}C NMR spectrum.

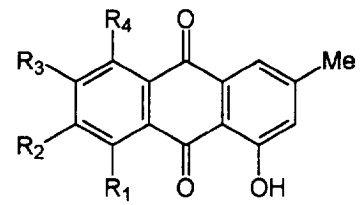
Calculation of the chemical shifts of the *peri*-hydroxyl protons using the data listed in Tables 1 and 2 yields values of δ 13.05 (1-OH) and 12.63 (5-OH) for 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**28**) and δ 12.28 (5-OH) and 12.08 (4-OH) for physcion (**29**). Kazmi *et al.* [15] reported δ 12.29 and 12.09, Lu *et al.* [16] δ 12.33 and 12.13, and Kelly *et al.* [17] δ 12.31 and 12.11 for **29**, and δ 12.93 and 12.53 for **28**.

Kazmi *et al.* [15] reported the ^{13}C NMR spectrum of the compound they isolated. It shows two carbonyl signals at δ 182.1 and 190.9, respectively. For a 1,5-dihydroxy substitution nearly identical values would be expected of *ca* δ 187, while for a 1,8 dihydroxysubstituted system values of *ca* δ 181 and 192 are expected [18]. There is thus no doubt that both Kazmi *et al.* [15] and Lu *et al.* [16] did isolate the previously known compound physcion (**29**).

Both Kazmi *et al.* [15] and Lu *et al.* [16] reported also the isolation of the compound ziganein (1,5-dihydroxy-3-methylanthraquinone, **30**). In their revision of the structure of przewalskinone B, Kelly *et al.* [17] did not mention this compound, but biosynthetic considerations make the occurrence of this compound similarly improbable. The occurrence of the 1,8-dihydroxy substituted isomer chrysophanol (1,8-dihydroxy-3-methylanthraquinone, **31**) would be much more likely. These two isomers can be distinguished in the same way as discussed above for physcion.

Calculation of the chemical shifts of the *peri*-hydroxyl protons using the data listed in Tables 1 and 2 yields values of δ 12.72 (1-OH) and 12.82 (5-OH) for ziganein (**30**) and δ 11.95 (1-OH) and 12.05 (8-OH) for chrysophanol (**31**). Kazmi *et al.* [15] reported δ 12.07 and 11.96, and Lu *et al.* [16] δ 12.13 and 12.01. Also, the ^{13}C NMR data reported by Kazmi *et al.* [15] are clear about the 1,8 disubstitution (δ 181.9 and 192.8). The melting point reported by Kazmi *et al.* [15] (191–193°) is similar to that reported for





	R_1	R_2	R_3	R_4
28	H	OMe	H	OH
29	OH	H	OMe	H

chrysophanol (195–196°) [19], while the melting point reported by Lu *et al.* [16 (227–228°) is identical to the previously reported literature value of ziganein [20]. Despite this melting point, there cannot be any doubt that not ziganein (**30**) but chrysophanol (**31**) was isolated from both *Salvia przewalskii* [16] and *Cassia italica* [15]. The occurrence of physcion and chrysophanol in these plants is also in accordance with the expected polyketide origin of these anthraquinones.

A considerable number of structures have been revised. Some concluding remarks can be made about the reasons why the anthraquinones were wrongly identified in the first place. In a number of cases, 1,8-dihydroxy substituted anthraquinones were identified as 1,5-dihydroxy substituted. This was always based on the wrong interpretation of the IR spectrum. Thus, despite the presence of two carbonyl bands, a strong one at 1620 and a weaker one at 1670 cm^{-1} [21], only the more intense band at 1620 cm^{-1} was taken into consideration.

Another reason has been the less cautious application of certain information. Beynon and Williams [22] found that in certain cases an $[M-18]^+$ fragment in the mass spectrum, due to the loss of water, could indicate the presence of a *peri*-methoxyl group. Later, Bowie and White [23] stated, after a more thorough investigation, that this loss of water also occurred when a methoxyl was located next to a hydroxyl, and thus one should be very careful in the interpretation. Despite the fact that Wijnsma and co-workers were aware of these facts [24] they used the loss of water from the molecular ion as a proof for a *peri*-methoxyl on various occasions [8, 9], leading in some cases to an erroneous identification. Their example was followed by other authors [10].

That NOE effects can give misleading results has been shown in this study. Anthraquinones usually have a M_r of *ca* 300. This means that due to the unfavourable molecular correlation times it will be difficult to obtain good NOE effects from these molecules. If a signal indicating a NOE effect is obtained one should always be aware that it could be an artefact or just not significant.

In this publication, the importance of the chemical shifts of *peri*-hydroxyl protons, measured in $CDCl_3$ for the structural elucidation of anthraquinones, has been demonstrated. These chemical shifts can also be measured in DMSO-d_6 and similar calculations to those performed in this publication should be possible. However, at the moment less data are available and the appropriate calculations need to be performed to obtain the new parameters.

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