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Deprotonation and Protonation of Hydroxyphenanthroperylenes

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Summary. Ground and excited state deprotonation and protonation pK_a values of hydroxyanthraquinones, hydroxyanthrones, hydroxyphenanthroperylenes, and the natural pigments hypericin and pseudohypericin were determined by means of spectrophotometric titrations and Förster cycle calculations. It was concluded that there is a strong intramolecular excited state proton transfer in the hydroxyanthraquinones and hydroxyanthrones due to a reversion of acidity and basicity of the hydroxyl and carbonyl groups in the excited state. However, in the hydroxyphenanthroperylene and the natural pigment excited states the order of basicity and acidity of these two functional groups remain unchanged. The site of deprotonation in hypericin and pseudohypericin was deduced by comparison between the pK_a values of suited model compounds and these pigments to be the hydroxyl group in position 3 or 4, respectively.

Keywords. Hypericin; Pseudohypericin; Hydroxyphenanthroperylenes; Protonation; Deprotonation; Spectrophotometric Titration; Ground state; Excited state.

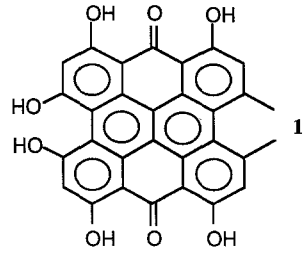
Deprotonierung und Protonierung von Hydroxyphenanthroperylenen

Zusammenfassung. Die Deprotonierungs- und Protonierungs-pK_a-Werte im Grundzustand und im angeregten Zustand von Hydroxyanthrachinonen, Hydroxyanthronen, Hydroxyphenanthroperylenen und den natürlichen Pigmenten Hypericin und Pseudohypericin wurde durch spektrophotometrische Titrationen und Förster-Zyklus-Rechnungen bestimmt. Bei den Hydroxyanthrachinonen und Hydroxyanthronen wurde auf Grund der Umkehr von Acidität und Basizität der Hydroxy- und Carbonylgruppen im angeregten Zustand auf einen starken intramolekularen Protonentransfer im angeregten Zustand geschlossen. Bei den Hydroxyphenanthroperylenen und den natürlichen Pigmenten bleiben die Aciditäts- und Basizitätsverhältnisse der beiden funktionellen Gruppen jedoch unverändert. Aus dem Vergleich der pK_a-Werte geeigneter Modellverbindungen mit Hypericin und Pseudohypericin wurde auf eine Deprotonierung der Hydroxylgruppe in Position 3 bzw. 4 geschlossen.

Introduction

On the one hand hypericin (**1**) constitutes the photodynamic agent contained in *Hypericum* species [1], on the other hand it is part of the protein – pigment system which governs photodynamic, i.e. photophobic, response in *Stentor* and related organisms [2]. In this latter respect the function of hypericin within its protein

environment was deduced to be that of a primary photodeprotonation system [2–4].



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Although deprotonation of **1** has been studied by means of absorption and fluorescence spectroscopy [3] in an aqueous system there is no detailed knowledge available on the site(s) of this reaction. Therefore it seemed to be interesting to assign the primary site of deprotonation and photodeprotonation as well as deriving the basicity of the carbonyl sites of **1**. The latter sites would be suited for photoprotonation, thus enabling the entire molecule to function as an excited state proton relay. This kind of information may be best deduced from a study of suited model compounds bearing hydroxyl groups at strategic positions.

Methods

The pK_a values of **1**, pseudohypericin (**12**), and the model compounds **2**–**11** were determined from spectrophotometric titrations [5]. A solvent mixture containing 80% dimethylsulfoxide in water was chosen for these investigations, as pure **1** is virtually insoluble in water. As was observed, titrations in water are disturbed by precipitation of **1** below *pH* values of about 7. Therefore such experiments yield apparent pK_a values which contain the solubility constant in addition to the dissociation constant. The separation of these two constants from an apparent pK_a value may become rather ambiguous. Tetrabutylammoniumhydroxide was used as the base indicating *pH* values by means of a glass electrode. At *pH* regions above 13 solutions of such a base (0.011 molar) result in defined H_- values on variation of the dimethylsulfoxide concentration [6].

Protonation was studied using aqueous sulfuric acid of various concentrations with defined H_0 values [7]. Excited state pK_a^* values were derived from ground state pK_a values and spectroscopic shifts for the lowest singlet transition ($\Delta\nu = \nu_{\text{neutral}} - \nu_{\text{protonation or deprotonation}}$) according to the Förster cycle: $pK_a^* = pK_a - 0.625 \cdot \Delta\nu \cdot T^{-1}$ [8].

Results and Discussion

Deprotonation

The results for the deprotonation of compounds **1**–**12** are shown in Table 1. To assign deprotonation of a certain hydroxyl group out of the six hydroxyl groups of hypericin (**1**), the simplest model systems of the hydroxyanthraquinone (**2**, **5**, **8**, **9**) and hydroxyanthrone (**3**, **6**, **10**) series were investigated. By formally "dimerizing" such systems the corresponding hydroxyphenanthroperylenediones **4**, **7**, and **11** are

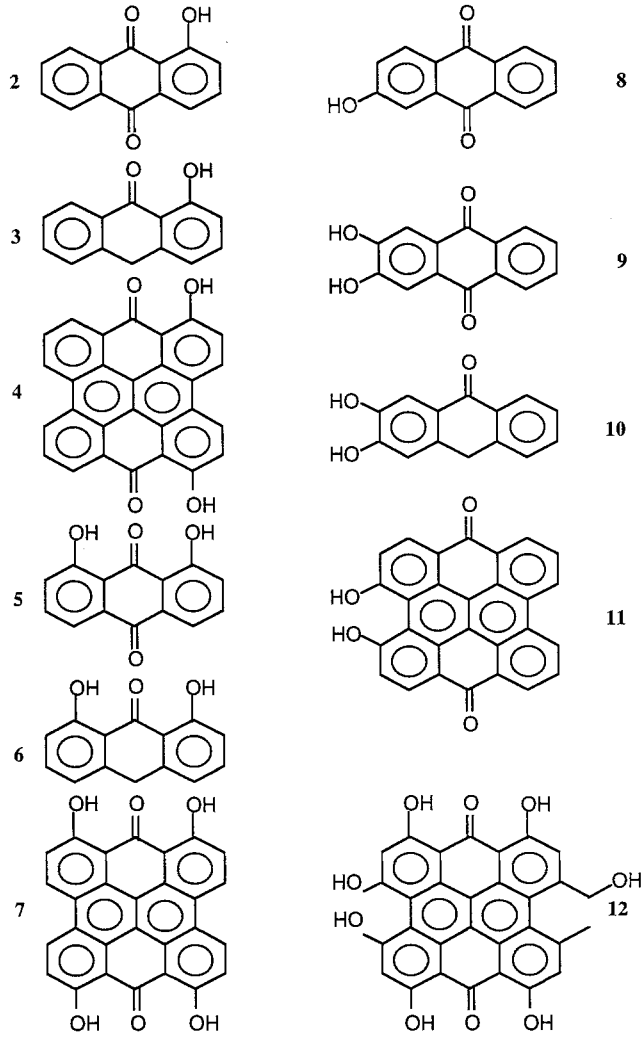

Table 1. Deprotonation of compounds **1**–**12** in the ground and excited states; solvent 80% dimethylsulfoxide in water, base tetrabutylammoniumhydroxide

Compound	λ (nm)	λ_- (nm)	$\varepsilon_\lambda/\varepsilon_{\lambda-}$	pK_a	pK_a^*
1	597	618	2.00	11.0	9.8
2	404	529	0.85	11.4	−0.8
3	470	530	0.10	12.2	7.2
4	502	589	0.87	14.0	7.8
5	430	538	1.07	10.5	0.7
6	480	542	0.51	13.0	8.0
7	557	630	0.30	13.0	8.6
8	377	506	0.47	10.0	−4.1
9	410	540	0.90	9.1	−3.2
10	340	396	0.61	11.0	2.3
11	497	593	1.07	12.0	5.2
12	598	627	2.63	11.3	9.7

formed, which serve as advanced models on the way to the natural pigments **1** and **12**. The ground state pK_a values of **1**–**12** obtained (Table 1) compare favorably with the values of substituted phenols (pK_a values about 10; water as the solvent) [9].

On proceeding from the 1-hydroxyanthraquinone **2** via the corresponding 1-hydroxyanthrone **3** to the "dimeric" system **4** (Table 1), it becomes evident that the anthrones are much better suited than the quinones to model the ground and excited state deprotonation behavior of the integral compound **4**. This holds also for the corresponding transition in the 3-hydroxy series: **8** or **9** via **10** to the integral system **11**. Acidity in the 1-hydroxy derivatives **2**, **3**, and **4** is consistently lower (by about two pK_a units in the ground state, and about three to five units in the excited state) than in the corresponding 3-hydroxy derivatives **8**–**11**. This effect is assigned to the stabilization of the 1-hydroxyl group by means of efficient hydrogen bonding to the adjacent carbonyl group.

Formal introduction of a second hydroxyl group into the systems **2**, **3** and **4** produces compounds **5**, **6**, and **7**. This change yields only small changes in the deprotonation behavior of corresponding compounds. The increase in acidity by about one pK_a unit on formal transformation of **4** into **7** may be due to a statistical effect. Comparing the acidities of **8** and **9** the intramolecular stabilization of the phenolate ion by hydrogen bonding with the *ortho* hydroxy group may be responsible for the higher acidity of **9**.

The ground state acidities of the natural pigments **1** and **12** are characterized by pK_a values in the region of 11 which is significantly closer to the value of 12.0 of the 3,4-dihydroxy compound **11** than to those of the 1-hydroxy derivatives **4** and **7** (pK_a values of 14.0 and 13.0; Table 1). Thus the first deprotonation step in hypericin (**1**) and pseudohypericin (**12**) has to be assigned to position 3.

It should be noted that in the di-, tetra-, and hexa-hydroxy compounds this first deprotonation step is followed by further ones. So, for example, **1** is further deprotonated at H_- values of about 14. This second deprotonation equlibrium is characterized by a broad absorption maximum at about 670 nm. However, the site of this deprotonation could not be unequivocally assigned from comparison with the model systems.

Protonation

Protonations of one of the carbonyl groups of **2**, **5**, **8**, and **9** are characterized by ground state pK_a values of −7.0, −8.1, −7.32, and −7.28 [10]. These data indicate a moderate enhancement of their basicity compared to their parent compound anthraquinone ($pK_a = -8.44$), which is attributed to the electron withdrawing effect of the hydroxyl group [10]. Estimated from the spectroscopic shifts given in Ref. [10] excited state pK_a values of these derivatives are calculated to be −0.5, 1.1, 5.5, and 4.4. The anthrone pK_a and pK_a^* values were found to be −5.02 and 6.15 [11]. However, the hydroxyanthrones **3**, **6**, and **10** seem to react when dissolved in the rather concentrated sulfuric acid water mixtures necessary for the pK_a determinations; thus no reliable pK_a values could be estimated for them. Nevertheless, in all the model systems a dramatic increase (seven to twelve orders of magnitude) of basicity of the carbonyl group in the excited state is observed.

Table 2. Protonation of compounds **1**, **4**, **7**, and **11** in the ground and excited states; solvent aqueous sulfuric acid

Compound	λ (nm)	λ_{H+} (nm)	$\varepsilon_\lambda/\varepsilon_{\lambda_{H+}}$	pK_a	pK_a*
1	597	650	0.05	−6.0	−3.2
4	507	587	0.61	−7.0	−1.4
7	545	639	0.18	−7.3	−1.7
11	505	590	0.25	−6.5	−0.5

The pK_a and pK_a* values of the "dimeric" systems **4**, **7**, and **11** (Table 2) indicate that this increase of excited state basicity is similar to those of the "monomers" **2** or **3**, **5** or **6**, and **8** or **9**, but appears to be dampened, especially with the non intramolecularly hydrogen bonded compounds **8**, **9**, and **11**. The "monomers" in this latter case exhibit rather high pK_a differences of about twelve orders of magnitude which makes them similar to anthrone (see above).

Excited State Proton Transfer

The possibility of excited state proton transfer, which has attracted much attention in recent years [8, 9, 12], can be derived immediately by comparison of ground and excited state protonation and deprotonation parameters. Two types of behavior may be traced for the systems investigated in this paper. They are exemplified by the "monomer" **5**, its excited state **5***, and the corresponding "dimer" **7** and its excited state **7*** in Fig. 1. Whereas in the first case the relative order of acidity and basicity of the hydroxyl and oxo groups are reversed, they retain their order in the second case. This allows for an excited state intramolecular proton transfer in the case of compounds like **5**; the latter has been investigated in detail by emission spectroscopy [13]. In compounds like **7** there is no reason for such an excited state proton transfer reaction. From a comparison of the acidity and basicity data in Tables 1 and 2, it follows that the natural pigment hypericin (**1**) will exhibit no

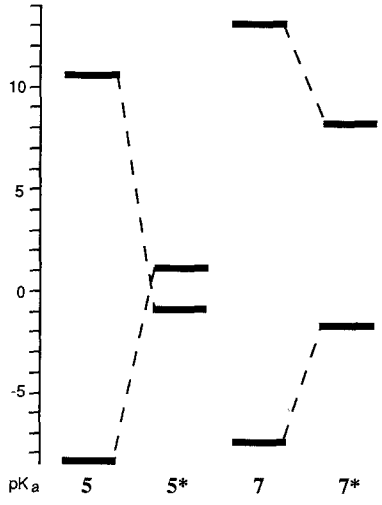

Fig. 1. Ground and excited state pK^a values for deprotonation and protonation of **5** and **7**

excited state intramolecular proton transfer which is due to a gap of nearly ten orders of magnitude between excited state acidity and basicity of the hydroxyl and carbonyl groups. Accordingly, **1** will only be deprotonated in the excited state by means of an intermolecular reaction with a suitable proton acceptor.

Experimental Part

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H-, IR-, UV-VIS-, and M-spectra and spectrophotometric titrations were recorded using the Bruker-WM-360-, and AC-200-, Biorad-FTS-45-, Hitachi-U-3210-, and Finnigan-MAT-115 instruments.

The pigments **1** and **12** were isolated from *Hypericum sp.* [14]. Compounds **2** and **3** were prepared according to [15, 16]. **5** was obtained from Aldrich. **8**, **9**, and **10** were prepared according to [17–19].

1,6-Dibenzoyldibenzo[a,o]perylene-7,16-dione [$C_{42}H_{22}O_6$]

62 mg (0.1 mmol) 1,6-dihydroxydibenzo[a,o]perylene-7,16-dione [20] were dissolved in 4 ml pyridine and 2 mg 4-dimethylaminopyridine were added as catalyst. The temperature was raised under agitation to about 70°C for 5 min., 28 mg (0.2 mmol) benzoylchloride was added dropwise and the resulting mixture was agitated for additional 3 h at room temperature. The reaction mixture was brought to *pH* 7 by addition of 2*N* HCl and extracted with dichloromethane. The organic layer was extracted with water, dried over Na_2SO_4 and evaporated. Chromatography (silica, dichloromethane) afforded 50 mg (80 %); m.p. 192 – 195°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 9.42 (d, 2 H, *J* = 9 Hz, CH-2,5), 8.26 (d, 4 H, *J* = 10.8 Hz, CH-2′,2″,6′,6″), 8.07 (d, 2 H, *J* = 7.6 Hz, CH-8,15), 7.95 (d, 2 H, *J* = 8.6 Hz, CH-3,4), 7.84 (dd, 4 H, *J* = 7.9 Hz, CH-11,12,4′,4″), 7.69 (t, 4 H, *J* = 7.2 Hz, CH-3′,3″,5′,5″), 7.54 (t, 2 H, *J* = 7.3 Hz, CH-9,14), 7.39 (t, 2 H, *J* = 7.3 Hz, -CH = 10,13) ppm. IR (KBr): ν = 1 750,1 640 cm^{-1}. UV-VIS (*DMSO*): λ = 424 (13 100), 405 (10 800), 466 (6 700) nm(ε). MS (70 eV, 350°C): *m/e* (%) = 622 (5; M^+), 518(3), 414(1), 298(1), 105(100), 77(25).

1,6-Dibenzoyl-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione [$C_{42}H_{20}O_6$]

80 mg (0.1 mmol) of the dibenzoperylene derivative described above was dissolved in 3 ml pyridine and irradiated at room temperature for 5 h using a 600 W tungsten lamp. The resulting precipitate was extracted three times with 5 ml portions of boiling pyridine and then with three portions of boiling benzene. Yield 60 mg (97%); m.p. 399°C, dec. IR (KBr): ν = 1 740, 1 650 cm^{-1}. UV-VIS (*DMSO*): λ = 424(8 025), 308(14 400), 259(29 500) nm (ε). MS (70 eV, 400°C): *m/e* (%) = 620(0.1; M^+), 516(0.5), 412(3), 298(1), 105(100) 77(25).

1,6-Dihydroxy-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione [**4**; $C_{28}H_{12}O_4$]

60 mg (0.15 mmol) of the dibenzoylphenanthroperylene derivative described above was suspended in 100 ml 40% aqueous NaOH and heated under reflux for 3 h. The resulting clear solution was acidified with 2*N* HCl, the precipitate centrifuged and washed three times with dist. water, methanol and chloroform. Yield 57 mg (92 %); m.p. > 400°C. IR (KBr): ν = 1 630 cm^{-1}. UV-VIS (*DMSO*): λ = 507(3 350), 421(3 030), 257(13 000) nm(ε). MS (70 eV, 280°C): *m/e* (%) = 410(10), 409(30; M^+), 408(14), 407(10), 307(17), 248(14), 247(100), 79(5), 69(1), 43(23), 39(28).

1,8-Dihydroxy-9(10 H)-anthrone [**6**; $C_{14}H_{10}O_3$]

2 g (8.84 mmol) **5** were solved in 20 ml conc. acetic acid, and 8 g (35.5 mmol) $SnCl_2 \cdot 2\,H_2O$ in 20 ml conc. HCl were added under an argon atmosphere. The reaction mixture was refluxed until **5** dis-

appeared (TLC control). The cold reaction mixture was extracted with dichloromethane, the organic layer washed with water to *pH* 7, dried over Na_2SO_4 and evaporated. Chromatography (silica, dichloromethane) afforded 1.8 g (90 %); m.p. 178°C, m.p. [21]: 178°C.

4,4',5,5'-Tetrahydroxy-1,1'-bianthraquinonyl [$C_{28}H_{14}O_8$]

60 mg (112 µmol) 4,4',5,5'-tetramethoxy-1,1'-bianthraquinonyl [22] were solved in 4.2 ml conc. acetic acid, 1 ml conc. H_2SO_4 was added, and the mixture was refluxed for 8 h. The cold reaction mixture was poured on ice, extracted with chloroform, the organic phase washed with water to *pH* 7, dried over Na_2SO_4 and evaporated. Chromatography (silica, chloroform) afforded 40 mg (75 %); m.p > 300°C. ^{1}H-NMR ($CDCl_3$, δ, 200 MHz): 12.6 (s, 2 H, OH), 12.0 (s, 2 H, OH), 7.62 (t, 2 H, J = 8 Hz, *ar*-H), 7.5 (AMX, 2 H, J_{AM} = 7.8 Hz, J_{MX} = 1.2 Hz, *ar*-H), 7.4 (s, 2 H, *ar*-H), 7.3 (s, 2 H, *ar*-H), 7.2 (AMX, 2 H, J_{AX} = 8.4 Hz, J_{MX} = 1.2 Hz, *ar*-H) ppm. IR (KBr): ν = 1 623 cm^{-1}. UV-VIS (ethanol) λ = 397(8 700) nm(ε).

1,6,8,13-tetrahydroxy-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione
[**7**; $C_{28}H_{12}O_6$]

50 mg (104 µmol) 4,4',5,5'-tetrahydroxy-1,1'-bianthraquinonyl described above was solved in 12 ml conc. H_2SO_4 and 50 mg freshly activated Cu [22] was added at room temperature. After the reaction mixture became green, it was decanted on ice. The precipitate was centrifuged, washed three times with ethanol and extracted three times with boiling chloroform. Yield 45 mg (98 %); m.p. > 320°C. IR (KBr): ν = 1 623 cm^{-1}. UV-VIS (*DMSO*): λ = 556(4 060), 422(4 020) nm(ε). MS (70 eV, 400°C): *m/e* (%) = 444(100; M^+), 415(6), 387(7), 371(7), 370(14), 296(5), 286(6), 285(10), 222(14), 208(5), 194(10), 171(12), 147(12), 146(8), 142(22), 141(12), 136(9), 129(5), 127(47), 126(41), 63(22), 62(6), 47(8), 44(27).

3,4-Dihydroxy-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione [**11**; $C_{28}H_{12}O_4$]

60 mg (0.15 mmol) 3,4-dihydroxy-dibenzo[a,o]perylene-7,16-dione [23] were solved in 500 ml benzene/methanol (1 / 1) and irradiated for 8 h with a TQ 150 Z2 lamp (Hanau). After evaporation of the solvent the residue is extracted three times with chloroform and five times with methanol. Yield 15 mg (25%); m.p. > 350°C. IR (KBr): ν = 1 622 cm^{-1}. UV-VIS (*DMSO*): λ = 475(6 600), 258(20 300) nm(ε). MS (70 eV, 280°C): *m/e* (%) = 411(1; M^+), 409(100), 392(2), 380(2), 363(2), 310(2), 247(6), 204(4), 79(3), 43(3), 39(9).

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References

[1] Roth L. (1990) Hypericum - Hypericin, Botanik-Inhaltsstoffe-Wirkung; ecomed, Landsberg
[2] Song P.-S. (1989) Molecular Electronics, Biosensors, and Biocomputers; Plenum Press, New York
[3] Walker E. B., Lee T. Y., Song P.-S. (1979) Biochim. Biophys. Acta **587**: 129
[4] Song P.-S. (1981) Biochim. Biophys. Acta **639**: 1

[5] Albert A., Serjeant E. P. (1984) The Determination of Ionization Constants; Chapman & Hall, London
[6] Stewart R., O'Donnell J. P. (1964) Can. J. Chem. **42**: 1681; Cox R. A., Stewart R. (1976) J. Am. Chem. Soc. **98**: 488
[7] Hammett L. P. (1973), Physikalische Organische Chemie. Verlag Chemie, Weinheim, S. 270
[8] Förster T. (1950) Z. Elektrochem. **54**: 43; Weller A. (1961) Progr. React. Kinet. **1**: 188
[9] Weller A. (1958) Z. Physikal. Chem. N. F. **15**: 438; Förster T. (1950) Z. Elektrochem. **54**: 531; Ireland J. F., Wyatt P. A. H. (1972) J. Chem. Soc. Faraday Trans. I **68**: 1053
[10] Gorelik M. V., Nesterova N. I., Mikhailova T. A., Kukushkina M. L. (1989) J. Org. Chem. USSR. **25**: 1849
[11] Stewart R., Granger M. R., Moodie R. B., Muenster L. J. (1963) Can. J. Chem. **41**: 1065; Hopkinson A. C., Wyatt P. A. H. (1967) J. Chem. Soc. **B 12**: 1333
[12] e. g. Strandjord A. S. G., Barbara P. F. (1985) J. Phys. Chem. **89**: 2355; a set of papers under the heading of "Spectroscopy and Dynamics of the Elementary Proton Transfer in Polyatomic Systems" (1989) Chem. Phys. 136; Kasha M. (1986) J. Chem. Soc. Faraday Trans. **82**: 2379
[13] Smulevich G., Foggi P., Feis A., Marzocchi M. P. (1987) J. Chem. Phys. **87**: 5664
[14] Falk H., Schmitzberger W. (Monatsh. Chem., in press)
[15] Bayer O. (1979) Houben Weyl **7/3c**: 102
[16] Steyermark L., Gardner J. E. (1930) J. Am. Chem. Soc. **52**: 4891
[17] analogous to [15]
[18] Bayer O. (1979) Houben Weyl **7/3c**: 96
[19] Attree G. F., Perkin A. G. (1931) J. Chem. Soc. **139**: 144
[20] Scholl R., Seer C. (1911) Ber. **44**: 1101
[21] Geiger W. (1974) Chem. Ber. **107**: 2976
[22] Brockmann H., Neeff E., Mühlmann E. (1950) Chem. Ber. **83**: 467
[23] Haller J. W. E., Perkin A. G. (1924) J. Chem. Soc. **125**: 236

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