Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Germany

Autoxidation of the antimicrobial alkaloid cleistopholine in solution

F. Dombeck, F. Bracher

Received August 8, 2005, accepted August 18, 2005

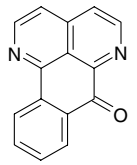
Prof. Dr. Franz Bracher, Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstr. 5–13, 81377 München, Germany
Franz.Bracher@cup.uni-muenchen.de

Pharmazie 61: 387–390 (2006)

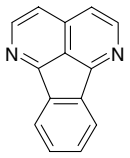
The alkaloid cleistopholine (**3**) undergoes rapid autoxidation in ethyl acetate solution under air giving rise to the corresponding *N*-oxide on one pathway, and to oxidation of the methyl group to a hydroxymethyl, formyl and further on to a carboxy group on the other one. Autoxidation can be suppressed by protection from light and air. In methanolic solution the extent of oxidation is less, in hydrochloric acid solution it is neglectable. The quinone moiety seems to be involved in the disposition to undergo oxidation.

1. Introduction

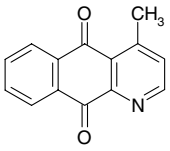
Aporphinoid alkaloids from Annonaceae (order Magnoliales) have attracted considerable interest because of their wide range of biological activities (Cavé 1987). In the past we performed considerable research on a small group of uncommon aromatic alkaloids from Annonaceae which are suggested to be biosynthesized from oxoaporphines by various degradation and nitrogen insertion reactions (Bracher 1992). Sampangine (**1**) is a azaoxoaporphine, eupolauridine (**2**), a formal decarbonylation product of **1**, has a diazafluoranthene ring system. Related tricyclic alkaloids are cleistopholine (**3**), a azaanthraquinone, and the azafluorenone onychine (**4**).



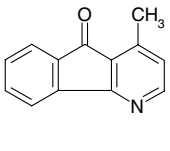
1



2



3



4

Our group and others (Bracher 1992) have worked out efficient total syntheses of the alkaloids mentioned above, that allowed screenings for biological activities. Alkaloids **1** to **4** and several substituted analogues thereof showed considerable antimicrobial activities with sampangine (**1**) and related azaoxoaporphines being the most active group (Bracher 1993, 1994; Hufford 1987; Peterson 1992; Tadic 1987). The azaoxoaporphines are most conveniently prepared from azaanthraquinones like **3** by a ring annellation method developed by us (Bracher 1989a). Hydroxy derivatives of cleistopholine (**3**) were shown to be active against Epstein-Barr virus, whereas the parent alkaloid **3** and methoxy derivatives were inactive (Konoshima 1989). In order to synthesize new antiviral azaanthraquinones and antimicrobial azaoxoaporphines, we further investigated transformations of readily available cleistopholine (**3**) (Bracher 1989a). In the course of this project we discovered unexpected autoxidations of **3** in solution.

2. Investigations and results

2.1. Chemistry

Storing an ethyl acetate solution of **3**, used as a TLC reference, resulted in the appearance of several new intensive spots on TLC within a few days. Since one less polar spot gave a bright yellow color on spraying with 3,5-dinitrophenylhydrazine reagent, we presumed that it might be an aldehyde. One very polar spot was supposed to be a carboxylic acid or a *N*-oxide. On the basis of behaviour on TLC we supposed that the decomposition products should arise from oxidation of the alkaloid. Therefore we first worked out syntheses of the putative oxidation products of **3** for unambiguous identification of the decomposition products by HPLC.
The *N*-oxide **5** was obtained by treating **3** with hydrogen peroxide in acetic acid. Oxidation of the methyl group of **3** with pyridinium chlorochromate in dichloromethane gave the aldehyde **6**. Further oxidation of **6** with hydrogen peroxide in formic acid gave the carboxylic acid **7** (Chaker 1997). Finally, aldehyde **6** was reduced to the primary alcohol **8** with sodium borohydride in methanol without affecting the quinone system (Scheme 1).

Scheme 1

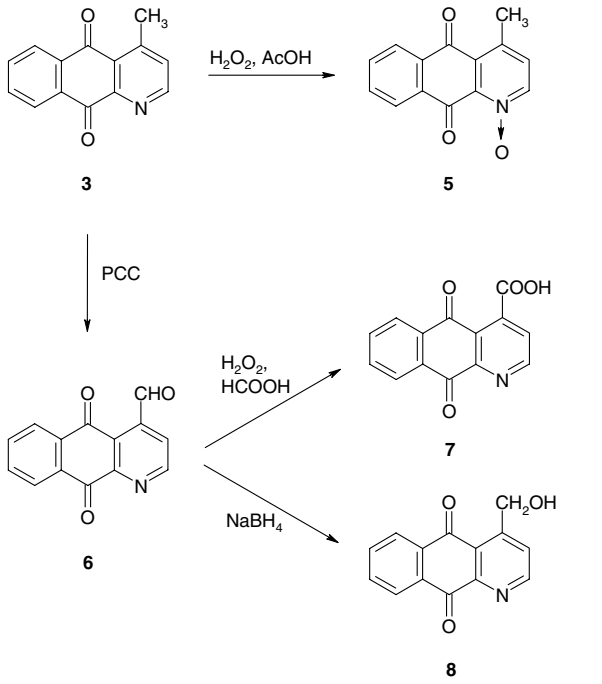


Table 1: Retention times of the reference compounds

Compound	Retention time [min]
7	1.34
5	6.76
6	8.30
8	8.97
3	16.60

2.2. Chromatographic system

A HPLC method that separates the alkaloid and the putative oxidation products (**5**, **6**, **7**, **8**) had to be developed in order to analyze the autoxidation of cleistopholine (**3**). This implicated that compounds with very different polarities had to be separated in a single run. First experiments using a RP-18 column (LiChrospher 100) with various eluents and flow rates were disappointing, since we did not obtain a separation of aldehyde **6** and alcohol **8**. Obviously, the polarity of the hydroxy group in **8** is significantly reduced by hydrogen bonding with the C-5 carbonyl group. Finally, a LiChrospher 60 RP-select B column, which is typically used for separation of basic compounds, was able to separate **6** and **7**. The retention times of the reference compounds are listed in Table 1.

2.3. Stability tests

Freshly prepared solutions of cleistopholine (**3**) in ethyl acetate (entry 1), methanol (entry 4) and 0.1 molar HCl (entry 5) were stored in glass flasks at room temperature, exposed to normal daylight. Additionally, a solution in ethyl acetate in a brown glass flask was stored under air (entry 2) and one other in a brown glass flask under nitrogen (entry 3).

Table 2 shows the compositions of the solutions after a period of 7 days, as determined by HPLC.

Obviously, extensive decomposition occurs in ethyl acetate solution under light exposition (entry 1). We found significant amounts of the aldehyde **6**. In addition small amounts of *N*-oxide **5**, carboxylic acid **7**, and a unidentified product (retention time 3.9 min; molecular mass 295) were detected, but not even traces of alcohol **8** could be found. Upon storage in a brown glass flask (entry 2), the extent of autoxidation was extremely reduced, and only traces of **5**, **6**, and **7** were found. Storing **3** in a brown glass flask under nitrogen gave almost no autoxidation products. In methanolic solution (entry 4) exposed to daylight, we found significantly lower contents of oxidation products compared to entry 1. Surprisingly, alcohol **8** could be detected in this experiment. In dilute hydrochloric acid (entry 5) we recorded almost no oxidation.

Table 2: Composition of solutions of cleistopholine (3) after 7 days (percentages based on integrations at 254 nm)

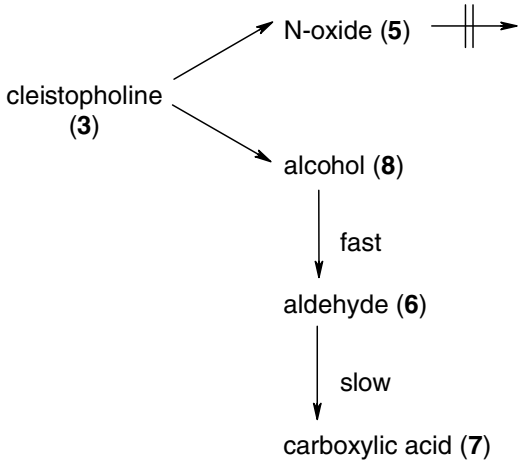
Entry	Solvent/conditions	Products				
		3	**5**	**6**	**7**	**8**
1	EtOAc/daylight[a]	39%	12%	31%	14%	–
2	EtOAc/brown glass[b]	96%	trace	2%	trace	–
3	EtOAc/brown glass[c]	99%	–	trace	–	–
4	MeOH/ aylight	79%	6%	trace	4%	9%
5	0.1 M HCl/daylight	98%	–	–	trace	

[a] about 4% of an unidentified product
[b] about 2% of an unidentified product
[c] under nitrogen

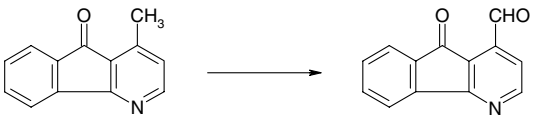
In order to get more insight into the decomposition of **3** in ethyl acetate solution, we also investigated the stabilities of the single oxidation products **5**–**8** in ethyl acetate under air upon exposure to air and daylight. The primary alcohol **8** was quantitatively oxidized to the aldehyde **6** (and minor amounts of carboxylic acid **7**) within a few days. Aldehyde **6** was slowly converted to the carboxylic acid **7**. Solutions of the *N*-oxide **5** and the carboxylic acid **7** did not contain any new products even after prolonged storage.

So one pathway of light-induced oxidation of cleistopholine (**3**) is formation of the *N*-oxide **5**. On the other pathway, the intermediate alcohol **8** is rapidly oxidized to the aldehyde **6**, which in turn is slowly further oxidized to the carboxylic acid **7**. The extent of oxidation strongly depends on the solvent. Protonation or oxidation of the ring nitrogen leads to products (hydrochloride, *N*-oxide) which show no tendency for autoxidation (Scheme 2).

Scheme 2



Scheme 3



4 9

Finally, we found that storage of the closely related azafluorenone alkaloid onychine (**4**) (Bracher 1989b) in ethyl acetate solution under air and daylight exposition resulted in the formation of only trace amounts of the corresponding aldehyde **9** (Scheme 3). An authentic sample of **9** was prepared by oxidation of onychine (**4**) using Vismara's method (Vismara 1987). This is a clear indication that the redox-active quinone system is involved in the autoxidation of the azaanthraquinone alkaloid cleistopholine (**3**).

2.4. Conclusion

In conclusion, solutions of cleistopholine (**3**) show extensive autoxidation when exposed to light and air. This autoxidation can be suppressed by exclusion of air and light, and by storing in acidic solution. The redox-active quinone system of **3** seems to be strongly involved in the oxidation reactions, since the realted azafluorenone onychine (**4**) is quite stable against autoxidation.

3. Experimental

3.1. General

Melting points were determined with a Büchi B-50 apparatus. IR spectra were run on a Perkin Elmer IR-881 infrared spectrometer as KBr plates. ^{1}H NMR spectra were recorded on Joel GSX 400 and Joel GSX 500 instruments. Chemical shifts are expressed in ppm with reference to TMS as an internal standard. Mass spectra were measured on a Hewlett Packard 5989 A mass spectrometer. Flash column chromatography was carried out on silica gel (Merck, Kieselgel 60). Analytical HPLC was performed with a Merck L 7100 pump with a Merck L 4000 UV detector and a LiChrospher® 60 RP-select B column (250 mm × 4 mm, Merck). Autoxidation experiments were performed with solutions in 100 ml Schott DURAN volumetric flasks. Results of elemental analyses were in an acceptable range througout.

3.2. Synthesis of the compounds

Cleistopholine (**3**) and onychine (**4**) were prepared as described earlier (Bracher 1989a, 1989b).

3.2.1. Synthesis of 4-methyl-1-oxy-benzo[g]quinoline-5,10-dione (cleistopholine-N-oxide) (**5**)

A solution of 223 mg (1.0 mmol) **3** in 10 ml acetic acid and 1 ml hydrogen peroxide (30%) was stirred at 75 °C. After 1 h 1 ml hydrogen peroxide solution (30%) was added and the mixture was stirred for another 2 h at 75 °C. The mixture was poured into ice-water (100 ml) and extracted with dichloromethane (3 × 40 ml). The combined organic layers were dried over Na_2SO_4, evaporated, and purified by column chromatography (ethyl acetate : hexane, 5 : 1) to give 100 mg (42%) of **5** as a orange solid; m.p. 169 °C. MS (70 eV): m/z 239 (2) [M^+], 223 (100), 166 (28), 119 (34). IR (KBr): (hydrochloride): ν_{max} = 1779, 1701, 1675, 1591, 1293 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 8.49 (d, J = 6.8 Hz, 1 H, 2-H), 8.09 (m, 2 H, 6-H, 9-H), 7.89 (m, 2 H, 7-H, 8-H), 7.61 (d, J = 6.8 Hz, 1 H, 3-H), 2.69 (s, 3 H, CH_3).
$C_{14}H_9NO_3$

3.2.2. Synthesis of 4-formyl-benzo[g]quinoline-5,10-dione (**6**)

A suspension of 120 mg (0.55 mmol) pyridinium chlorochromate, 1.0 g magnesium sulphate, and 112 mg (0.50 mmol) cleistopholine (**3**) in 8 ml dichloromethane was stirred vigorously for 15 h, then treated with 40 ml ethyl acetate and filtered through a thin pad of silica. The filtrate was evaporated and the residue purified by column chromatography (ethyl acetate : hexane, 1 : 1) to give 45 mg (38%) of **8** as a brownish-yellow solid; m.p. 237 °C. MS (70 eV): m/z 237 (15) [M^+], 209 (100), 181 (42), 153 (72), 126 (31), 76 (26). IR (KBr): ν_{max} = 1698, 1685, 1660, 1588, 1298, 1254 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.88 (s, 1 H, CHO), 9.27 (d, J = 4.7 Hz, 1 H, 2-H), 8.44 (dd, J = 6.3, 1.8 Hz, 1 H, 9-H), 8.31 (dd, J = 6.3, 1.8 Hz, 1 H, 6-H), 7.91 (m, 2 H, 7-H, 8-H), 7.88 (d, J = 4.7 Hz, 1 H, 3-H).
$C_{14}H_7NO_3$

3.2.3. Synthesis of 4-carboxy-benzo[g]quinoline-5,10-dione (**7**)

A solution of 1.90 g (8.0 mmol) **6** in 40 ml formic acid was cooled to 0 °C and then treated with 8 ml hydrogen peroxide solution (30%) with stirring. After 2 h additional 5 ml hydrogen peroxide solution (30%) were added and the mixture was stirred for 15 h at ambient temperature. The precipitate was collected by filtration, washed with water and cold ethyl acetate, and dried at 120 °C for 1 h to give 1.86 g (92%) of a light brown solid; m.p. 316 °C (dec.) (Chaker 1997: m.p. >300 °C). MS: m/z 253 (6) [M^+], 209 (100), 181 (60), 153 (58), 126 (21), 125 (14). IR (KBr): ν_{max} = 3421, 1713, 1685, 1660, 1590, 1543, 1464, 1391, 1302, 1154, 982, 864, 726, 687 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 13.78 (br. s, 1 H, COOH), 9.13 (d, J = 4.9 Hz, 1 H, 2-H), 8.24 (dd, J = 6.3, 1.8 Hz, 1 H, 9-H), 8.17 (dd, J = 6.3, 1.8 Hz, 1 H, 6-H), 7.96 (m, 2 H, 7-H, 8-H), 7.86 (d, J = 4.9 Hz, 1 H, 3-H).
$C_{14}H_7NO_4$

3.2.4. Synthesis of 4-hydroxymethyl-benzo[g]quinoline-5,10-dione (**8**)

Sodium borohydride (76 mg, 2.0 mmol) was added to a suspension of 119 mg (0.50 mmol) **6** in 8 ml methanol and the mixture was stirred for 1 h. The mixture was evaporated and the residue treated with brine and extracted with ethyl acetate (2 × 100 ml). The combined organic layers were dried over Na_2SO_4, evaporated, and purified by column chromatography (ethyl acetate : hexane, 2 : 1) to give 102 mg (85%) of **8** as a brownish-yellow solid; m.p. 192 °C. MS (70 eV): m/z 239 (82) [M^+], 223 (93), 210 (44), 209 (44), 195 (47), 167 (63), 149 (100). IR (KBr): ν_{max} = 3308, 1681, 1662, 1590, 1300, 720 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 9.09 (d, J = 4.8 Hz, 1 H, 2-H), 8.39 (m, 1 H, 9-H), 8.28 (m, 1 H, 6-H), 7.98 (d, J = 4.8 Hz, 1 H, 3-H), 7.85 (m, 2 H, 7-H, 8-H), 5.25 (s, 2 H, CH_2). 3.19 (br. s, OH).
$C_{14}H_9NO_3$

3.2.5. Synthesis of 4-formyl-5-oxo-5H-indeno[1,2-b]pyridine (**9**)

Onychine (**4**) (195 mg, 1.0 mmol) was dissolved in 10 ml DMSO and treated with 127 mg (1.0 mmol) iodine, 37 mg (0.2 mmol) *tert.*-butyl iodide, 12 mg (0.06 mmol) ferrous chloride tetrahydrate, and 137 mg (1.2 mmol) trifluoroacetic acid. The mixture was heated at 90 °C for 6 h and then poured into 80 ml ice-water. After addition of 150 ml sodium thiosulphate solution (20%) the mixture was adjusted to pH 9 with sodium bicarbonate solution and extracted with dichloromethane (2 × 70 ml). The combined organic layers were washed with 200 ml water, dried over Na_2SO_4, evaporated, and purified by column chromatography (ethyl acetate:hexane, 1 : 1) to give 192 mg (92%) of **9** as a yellow solid; m.p. 165 °C. MS (70 eV): m/z 209 (31) [M^+], 181 (100), 153 (62), 126 (35), 99 (9). IR (KBr): ν_{max} = 1710, 1604, 1562, 1395, 1243, 1233, 754 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 11.01 (s, 1 H, CHO), 8.75 (d, J = 5.3 Hz, 1 H, 2-H), 7.89 (d, J = 7.4 Hz, 1 H, 9-H), 7.76 (d, J = 7.4 Hz, 1 H, 6-H), 7.67 (td, J = 7.4, 1.0 Hz, 1 H, 8-H), 7.57 (d, J = 5.3 Hz, 1 H, 3-H), 7.50 (td, J = 7.4, 1.0 Hz, 1 H, 7-H).
$C_{13}H_7NO_2$

3.3. Stability tests

Cleistopholine (**3**; 20 mg) was dissolved in 100 ml of the solvent (see Table 2) in a 100 ml volumetric flask. The flask was stoppered and stored at ambient temperature near the window of the laboratory. The sample under N_2 (entry 3) was prepared by bubbling a stream of N_2 through the solution of **3** in ethyl acetate under ultrasound irradiation for about 3 min, and then stoppering the flask. Aliquots for HPLC analysis were taken and were directly injected to the HPLC (injection volume: 20 µl). HPLC analysis was performed on a LiChrospher® 60 RP-select B column with methanol : water (50 : 50) as the eluent at a flow of 1.0 ml/min with UV-detection at 254 nm.

Acknowledgements: The authors are grateful to the Fonds der Chemischen Industrie, Frankfurt, for financial support, and to Florian Lirk and Michael Baltrusch for technical support.

References

Bracher F (1989a) Synthese von Cleistopholin und Sampangin. Liebigs Ann Chem 87–88.
Bracher F (1989b) Synthese von Onychin und Eupolauridin. Arch Pharm (Weinheim) 322: 293–294.
Bracher F (1992) Annonaceen-Alkaloide mit neuartigen Grundgerüsten – biologische, chemische und pharmakologische Aspekte. Pharm Ztg Wiss 5: 109–117.

Bracher F (1993) Polycyclische aromatische Alkaloide, 9. Mitt.: Synthese und antimykotische Aktivität von Diazafluoranthen-Alkaloiden. Pharmazie 48: 521–523.

Bracher F (1994) Annonaceen-Alkaloide mit antimykotischer Aktivität. Arch Pharm (Weinheim) 327: 371–375.

Cavé A, Leboef M, Waterman PG (1987) The aporphinoid alkaloids of the annonaceae. Alkaloids, Chem. Biol. Perspect. Vol. 5, Wiley Interscience Publ., New York, pp. 133–270.

Chaker L, Pautet F, Fillion H (1997) Synthesis of 2*H*-benzo[2,3-*g*]pyridazino[4,5-*d*,*e*]quinolin-3-one derivatives. Monatsh Chem 128: 681–685.

Hufford CD, Liu S, Clark AM (1987) Anticandidal activity of eupolauridine and onychine, alkaloids from Cleistopholis patens. J Nat Prod 50: 961–964.

Konoshima T, Kozuka M, Koyama J, Okatani T, Tagahara K, Tokuda H (1989) Studies on inhibitors of skin tumor promotion, VI. Inhibitory effects of quinones on Epstein-Barr virus activation. J Nat Prod 52: 987–995.

Peterson JR, Zjawiony JK, Liu S, Hufford CD, Clark AM, Rogers RD (1992) Copyrine alkaloids: Synthesis, spectroscopic characterization, and antimycotic/antimycobacterial activity of A- and B-ring-functionalized sampangines. J Med Chem 35: 4069–4077.

Tadić D, Cassels BK, Leboeuf M, Cavé A (1987) Kinabaline and the aporphinoid biogenesis of azaanthracene and azafluorene alkaloids. Phytochemistry 26: 537–541.

Vismara E, Fontana R, Minisci F (1987) A new method for the oxidation of methyl to formyl groups in heteroaromatic derivatives. Gazz Chim Ital 117: 135–136.