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Chemical Stability of the Hagedorn Oximes HGG-12 and HI-6

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The chemical stability of the soman antidotes HGG-12 and HI-6 was studied over the pH range 2–9 at various temperatures. Maximum stability for both oximes was found at pH 2. From *Arrhenius* plots the predicted shelf life (10% decomposition) was 2.6 years at 25 °C and 60 years at 4 °C for both oximes. The apparent energy of activation was 113 kJ/mol. The decay rate of both oximes apparently was not influenced by oxime concentration, buffer composition, light, and oxygen. By ion-pair HPLC the following degradation products were indentified: pyridine-2-carbaldoxime, pyridine-2-carbonitrile, 2-pyridone, formaldehyde and 3-benzoylpyridine (for HGG-12) or isonicotinamide and isonicotinic acid (for HI-6). Two additional pyridinium compounds have not yet been identified. The pattern of degradation products varied considerably with pH.

Die chemische Stabilität der Hagedorn-Oxime HGG-12 und HI-6

Die chemische Stabilität der Soman-Antidote HGG-12 und HI-6 wurde im pH-Bereich zwischen 2 und 9 untersucht. Beide Oxime waren bei pH 2 am stabilsten. Die Beständigkeit beider Oxime (10% Zerfall) wurde nach der *Arrhenius*-Gleichung mit 2,6 Jahren bei 25°, mit 60 Jahren bei 4° errechnet. Die scheinbare Aktivierungsenergie des Zerfalls betrug 113 kJ/mol. Die Zerfallsgeschwindigkeit beider Oxime scheint weder von der Oximkonzentration, noch von der Pufferzusammensetzung, von Licht oder von Sauerstoff abzuhängen.

Durch Ionen-Paar HPLC wurden folgende Zerfallsprodukte identifiziert: 2-Pyridincarbaldoxim, 2-Cyanopyridin, 2-Pyridon, Formaldehyd und 3-Benzoylpyridin (HGG 12) bzw. Isonicotinamid und Isonicotinsäure (HI-6). Zwei weitere Pyridinium-Verbindungen wurden bisher noch nicht identifiziert. Das Muster der Zerfallsprodukte ist stark pH-abhängig.

The generally accepted mechanism by which organophosphorus agents cause toxic effects is through phosphorylation or phosphonylation of acetylcholinesterase[1]. During the past two decades, several bis-pyridinium oximes have been developed in the laboratory of I. Hagedorn (Freiburg, F.R.G.), among which obidoxime (Toxogonin®) has proved its superiority in reactivating acetylcholinesterase blocked by insecticides like paraoxon[2]. The failure of obidoxime, however, to reactivate significantly acetylcholinesterase blocked by soman[3] and the failure to reverse the fatal outcome after soman poisoning[4] has prompted several research groups to synthesize other pyridinium oximes in order to fill this therapeutic gap. Of the various compounds tested, HGG-12 and HI-6 (formulae see Fig. 1) were reported to exhibit some promising properties in soman poisoning[4–8]. Part of the beneficial effects may be due to reactivation of the inhibited acetylcholinesterase, especially in the case of HI-6[8–10]. In addition, both oximes have ganglion blocking properties[11,12] and

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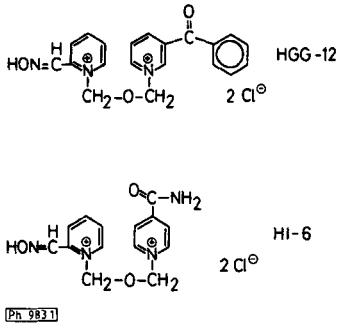

Fig. 1: Structural formulae of HGG-12, (3-benzoylpyridinium (1)methyl)-(2'-hydroxyiminomethylpyridinium(1')methyl)ether dichloride), and HI-6 (4-carboxamidopyridinium (1)methyl)-(2'hydroxyiminomethylpyridinium(1')methyl)ether dichloride).

antimuscarinic effects with HGG-12 being the more potent[13]. Therefore, it ist difficult to attribute the observed beneficial effects to a distinct reaction mechanism[4,14-16]. From the published data available, HI-6 appears at present as the most promising antidote against soman poisoning, at least in rats and mice[15,16]. Unfortunately, this compound was reported to be quite unstable in solution (*J.G. Clement*, personal communication, 1983). For HGG-12 no stability data exist at present. Hence, it seemed urgent to study the kinetics and products of the decomposition of both oximes in solution.

Detailed studies on the chemical stability of some bispyridinium aldoximes linked by a dimethylether bridge, especially obidoxime, were performed by *Christenson*[17,18]. In the case of HS-6, the 3-carboxamido isomer of HI-6, nicotinamide, formaldehyde, 2-pyridine-carbaldoxime and "a compound with absorbance at the same wavelength as HS-6" were reported[19]. This notice called for some caution in establishing appropriate methods for the determination of intact oximes. Quantitative determination of bispyridinium oximes is usually performed by photometry at 350 nm in slightly alkaline media. Though sensitive, this method is quite unspecific and may be disturbed by biological material or decomposition products of the oximes. Therefore, we developed a HPLC method for the specific detection of HGG-12 and HI-6 and their decomposition products. Because of the high hydrophilicity of these quaternary pyridinium compounds we applied ion-pair chromatography on reversed-phase material. Sufficient resolution was achieved with a mobile phase consisting of methanol and heptane sulfonate buffered with acetic acid. Similar systems had been recently applied by *Brown* et al.[20] and *Benschop* et al.[21].

Our experiments show that the chemical stabilities of HGG-12 and HI-6 are very similar between pH 2 and 7. Both oximes are most stable around pH 2 with apparent energies of activation of 113 kJ/mol. This value agrees with the data of *Christenson*[19] in the case of the uncatalyzed hydrolysis of HS-6, the para-isomer of HI-6 (115 kJ/mol). From the *Arrhenius* plot (Fig. 2) the apparent first-order rate constant for HGG-12 and HI-6 is about 5×10^{-6} h^{-1} at pH 2 and 25 °C, which is lower than for obidoxime, 10^{-3} h^{-1} [17], but equal to HS-6[19]. The predicted shelf life (10 % decomposition) for HGG-12 and HI-6 is about 2.6 years at 25° and 60 years at 4 °C. Because of the high energy of activation of the degradation, solutions of both oximes should be stored at rather low temperatures. Interestingly, the maximal stability of both oximes was observed below pH 3, whereas HS-6 had its maximal stability around pH 3[19] and obidoxime around pH 5[17].

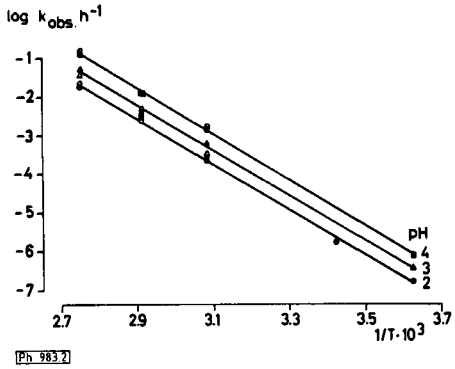

Fig. 2: *Arrhenius* plot of the decomposition rate constants of HGG-12 (closed symbols) and HI-6 (open symbols) at pH 2, 3, and 4. The rate constants $k_{obs.}$ were calculated from fitted exponential functions of the data in the tables 1 and 2.

The pattern of decomposition products of both oximes is similar to that observed with pralidoxime, obidoxime and HS-6[18]. At low pH, the acetal bridge is formally hydrolyzed giving rise to formaldehyde, 2-pyridine-carbaldoxime and 3-benzoylpyridine or isonicotinamide, respectively. At neutral pH, the pattern of decomposition is more complicated: Primary attack seems to be at the oxime (probably the oximate) group with formation of as yet unidentified bis-pyridinium compounds. Since 2-cyanopyridine and 2-pyridone have been detected in incubates of both oximes at pH 7.4, transient nitrile formation can be assumed. Identification of these degradation products and investigation of their possible biological activity is under current study.

Experimental Part

HGG-12 dichloride, MW 420.3, was synthesized by Merck, Darmstadt. The product contained 11.0 % crystal ethanol and 1 % crystal water (w/w). A total of less than 0.2 % impurities was detected by HPLC, of which 3-benzoylpyridine made 0.1 %. For calculations, an apparent MW of 479 was used. *HI-6 dichloride* monohydrate, MW 377.23, was a generous gift from *J.G. Clement*, Defense Research Establishment Suffield, Ralston Alberta, Canada. The compound was pure as judged from elemental analysis and HPLC.

Isonicotinamide and isonicotinic acid were obtained from EGA-Chemie, Steinheim F.R.G.; 3-benzoylpyridine, 2-pyridine-carbaldoxime, 2-cyanopyridine, 2-pyridone and all other chemicals were from Merck, Darmstadt.

HPLC: chromatograph ALC/GPC 244 (Waters, Milford, MA) on μ-Bondapak C_{18} (4 mm int. diam. × 30 cm; flow rate 1.5 ml/min; detection at 254 nm; peak integration by a Data Module®, Waters). Mobile phase: MeOH/H_2O/heptanesulfonate, buffered with acetic acid to pH 3.2 (PIC-B_7 reagent®, Waters).

With MeOH/H_2O/PIC-B_7 = 38:60:2, 2-pyridine-carbaldoxime was eluted after 4.6 ml, 3-benzoylpy-

ridine after 13 ml and HGG-12 after 21 ml. With $MeOH/H_2O/PIC\text{-}B_7$ = 20:78:2, isonicotinic acid was eluted after 3.4 ml, 2-pyridone after 4.6 ml, isonicotinamide after 5.5 ml, 2-cyanopyridine after 6.5 ml, 2-pyridine-carbaldoxime after 8.4 ml, and HI-6 after 30 ml. This low methanol content resulted in considerable tailing of HI-6 as shown in Fig. 4, but was necessary to obtain sufficient separation from minor degradation products.

Thin layer Chromatography (TLC): silica gel 60 F_{254} plates, 0.2 mm layer (Merck, Darmstadt) with chloroform/methanol (90:10). The RF-values were: 2-cyanopyridine = 0.9; 3-benzoylpyridine = 0.86; 2-pyridine-carbaldoxime = 0.63; 2-pyridone = 0.48; isonicotinamide = 0.36; isonicotinic acid = 0.05. With chloroform/methanol = 95:5, the RF-value for formaldimethone was 0.65.

Formaldehyde was determined spectroscopically as formaldimethone.

Rates of Decomposition

Solutions of HGG-12 and HI-6 were incubated in 0.1 M-sodium phosphate or pyrophosphate at various pH values and temp. All solutions were kept under nitrogen in sealed glass ampoules. Tables 1 and 2 show the percentage of residual oxime as determined by HPLC. Analysis of the time-dependent degradation revealed first-order kinetics at all pH-values and temp. chosen. The rate of degradation was apparently independent of the oxime conc. (1×10^{-4} up to 1×10^{-1} M) and was not influenced by light and oxygen. The data of the residual HGG-12 at 20° and 4°C were calcd. from the difference of the initial HGG-12 conc. and the conc. of 3-benzoylpyridine formed. This degradation product is directly proportional to the amount of degraded HGG-12 at pH 2–4 (see below).

From the data in tables 1 and 2, the observed first-order rate constants were calcd. for the *Arrhenius* plot presented in Fig. 2. From these data it is evident that the stability of HGG-12 and HI-6 is identical between pH 2 and 7. At alkaline pH, HI-6 seems to be slightly more stable, but the difference is marginal (Fig. 3).

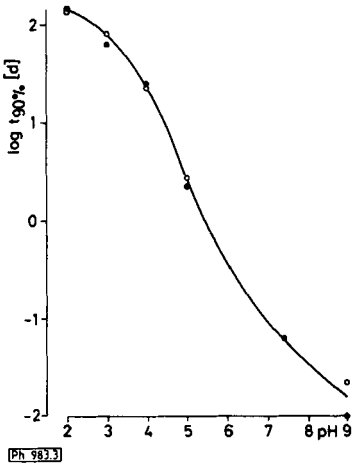

Fig. 3: Plot of the estimated shelf lives (10 % decomposition) of HGG-12 and HI-6 at 37°C vs. pH. The shelf lives were taken from the data of the tables 1 and 2.
(Open symbols = HGG-12, closed symbols = HI-6).

Table 1: *Decomposition of HGG-12 at Various pH and Temperatures*

Temp. (°K)	Time of incubation (hr)	pH 2	pH 3	pH 4	pH 5	pH 7.4	pH 9
		(percentage of residual HGG – 12)					
	1	98.7	97.9	n.d.			
	2	97.9	92.1	78.4			
364	4	94.3	83.0	45.3			
	6	91.8	75.8	41.4			
	12.5	80.5	51.9	19.7			
	24	65.7	28.9	3.4			
	2	102.9	100.5	93.4			
344	4	98.8	97.8	92.7			
	24.5	93.0	90.3	63.8			
	48	88.4	83.1	45.2			
	24	94.8	93.3	94.4			
324	72	91.6	90.5	84,3			
	168	n.d.	85.0	75.5			
	336	87.7	n.d.	60.3			
	0.33				n.d.	n.d.	87.5
	1				n.d.	94.3	66.8
	2				n.d.	86.0	41.9
	4				98.7	71.6	17.2
	6				98.4	64.3	7.9
	8				n.d.	n.d.	3.5
310	10				100.4	49.2	n.d.
	14				n.d.	31.4	n.d.
	30				96.0	12.6	n.d.
	54.1				92.0		
	94.5				85.6		
	117				78.3		
	141				79.6		
	168				70.0		
	960	99.79					
293	2544	99.70					
	10944	98.03					
	17112	97.00					
	960	100.02	99.99	100.0			
277	2544	99.95	99.87	99.69			
	10944	99.83	99.58	99.13			

HGG-12 (1 mM) dissolved in 0.1 M-sodium phosphate or pyrophosphate (pH 9) was incubated in sealed glass ampoules under nitrogen. The samples stored at 277° and 293° K were dissolved in 50 mM-sodium citrate. The data of the residual HGG-12 from these latter samples were calculated from the difference of initial HGG-12 and the amount of 3-benzoylpyridine formed.

n.d. = not determined.

Table 2: *Decomposition of HI-6 at Various pH and Temperatures*

Temp. (K°)	Time of incubation (hr)	pH 2	pH 3	pH 4	pH 5	pH 7.4	pH 9
		(percentage of residual HI – 6)					
	1	101.8	96.9	85.5			
	2	99.2	95.5	66.9			
364	4	96.3	88.4	40.6			
	6	91.9	86.0	32.1			
	19.2	66.9	49.3	3.7			
	25	61.7	41.1	1.4			
	2	98.2	96.4	93.5			
	4	98.7	98.3	94.9			
344	24.5	93.8	89.8	66.6			
	48	87.5	79.6	41.7			
	24	100.4	99.9	100.7			
324	72	96.7	96.1	89.8			
	168	93.0	92.8	76.0			
	336	92.7	90.3	60.9			
	0.5				99.3	99.7	98.3
	1				98.7	96.3	86.1
	2				99.9	89.2	69.6
310	4				101.5	77.2	46.1
	7				n.d.	63.9	25.9
	24				96.5	18.3	0.8
	48				92.7	3.4	0.0

HI-6 (1 mM) dissolved in 0.1 M-sodium phosphate or pyrophosphate (pH 9) was incubated in sealed glass ampoules under nitrogen.

Identification of Reaction Products

Formaldehyde as formaldimethone

HGG-12 (35 µmol) dissolved in 10 ml of 0.2 M-sodium phosphate, pH 6.3, was mixed with 50 mg dimedone and allowed to react under stirring at ambient temp. for 24 h. The incubate was extracted twice with n-hexane (v/v). On evaporation of the hexane-phase the reaction product crystallized. After re-crystallization the compound melted between 191 and 192° (formaldimethone, 191–191,5°,[22]). The mixture m.p. with authentic formaldimethone was 191°. The isolated compound was further identified by comparison with an authentic specimen (UV, TLC, HPLC). From 35 µmol HGG-12, 7.4 mg = 27 µmol formaldimethone was isolated.

2-Pyridine-carbaldoxime and 3-benzoylpyridine

HGG-12 (1.75 mmol) dissolved in 500 ml of 0.2 M-sodium phosphate, pH 6.3, was allowed to decompose at ambient temp. for 3 days. The incubate was extracted twice with ether (v/v). From the etheral phase some material was re-extracted with 0.1 M-NaOH as revealed by UV-spectroscopy.

a) After neutralization this material was extracted again with ether. Following drying over anhydrous sodium sulfate the ether was evaporated with precipitation of white needles. After recrystallization from ether the product melted at 112° (2-pyridine-carbaldoxime, 110–112°, Aldrich Catalogue). The mixture m.p. with authentic 2-pyridine-carbaldoxime was 112°- The identity of the isolated compound with 2-pyridine-carbaldoxime was confirmed by UV, TLC and HPLC. From 1.75 mmol HGG-12, 20 mg = 0.15 mmol 2-pyridine-carbaldoxime was isolated.

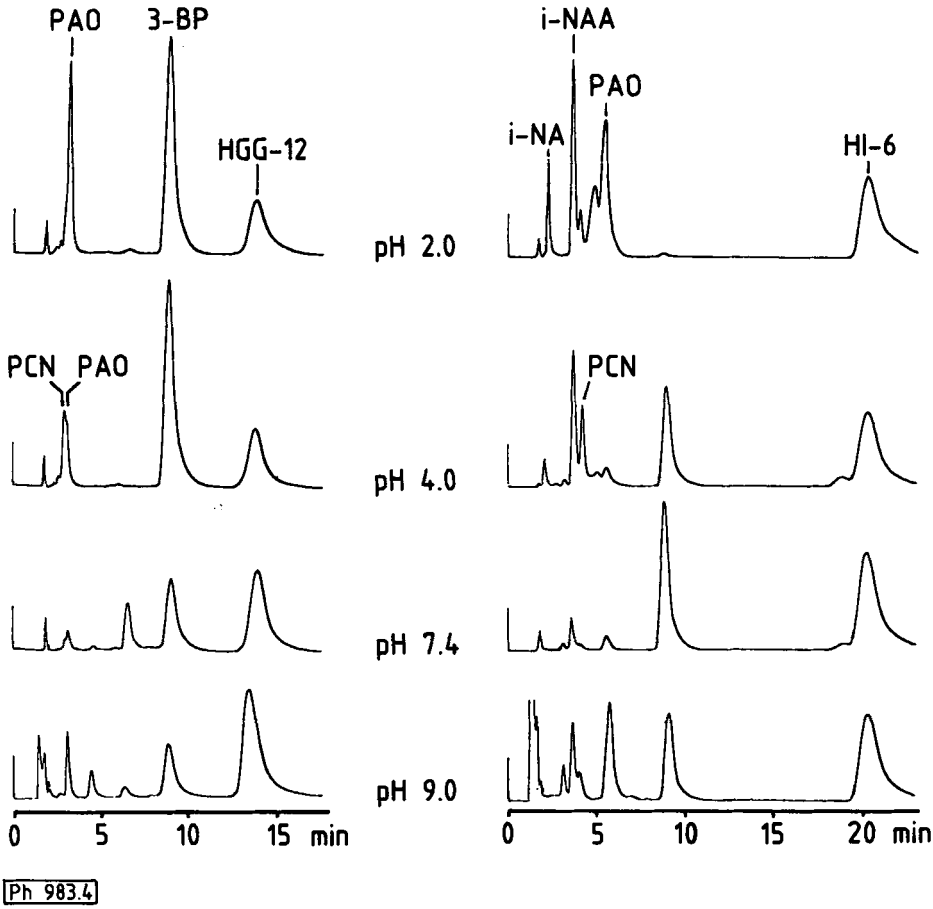

Fig. 4: HPLC of decomposition products of HGG-12 (left) and HI-6 (right), incubated at various pH and temp. Temp. and reaction times were selected to achieve about 60 % decomposition of the oximes at all pH values. Oximes (1 Mm) were dissolved in 0.1 M-sodium phosphate or pyrophosphate (pH 9). At pH 2, the oximes reacted at 92° for 60 h; at pH 4 at 92° for 8 h, at pH 7.4 at 37° for 15 h, and at pH 9 at 37° for 5 h. The chromatography was performed on μ-Bondapak C_{18} with 38 % MeOH or 20 % MeOH for HGG-12 and HI-6, respectively; see methods. PAO = 2-pyridine-carbaldoxime; 3-BP = 3-benzoylpyridine; PCN = 2-cyanopyridine; i-NA = isonicotinic acid; i-NAA = isonicotinamide.

b) The etheral phase of the above HGG-12 solution after re-extraction with sodium hydroxide was extracted with 0.1 M-HCl. After neutralization UV-absorbing material was extracted with ether. After drying over anhydrous sodium sulfate the ether was evaporated, resulting in an oily residue which solidified in the cold. The compound was identified as 3-benzoylpyridine by UV, TLC, and HPLC. From 1.75 mmol HGG-12, 75 mg = 0.41 mmol 3-benzoylpyridine was isolated.

Formation of 2-pyridine-carbaldoxime and 3-benzoylpyridine as decomposition products of HGG-12 was recently described by *Gross*[23].

Dependence of the degradation pathway on pH

Analysis of the degradation products of HGG-12 by HPLC revealed a remarkable dependence of the pattern of products on pH, which is illustrated in Fig. 4. At pH 2, HGG-12 decomposes mainly into 3-benzoylpyridine, 2-pyridine-carbaldoxime and formaldehyde. At pH 4, some additional 2-cyanopyridine was detected (UV, TLC, HPLC). At pH 7.4, the amounts of 3-benzoylpyridine, 2-pyridine-carbaldoxime and formaldehyde decreased in favour of as yet unidentified products that were not extractable with ether and yielded 2-pyridone and 3-benzoylpyridine upon acidification. These degradation products point to primary attack at the oxime group.

A similar degradation pathway was also observed with HI-6: At pH 2, isonicotinic acid and isonicotinamide (HPLC, TLC, UV) corresponded to the amount of 2-pyridine-carbaldoxime and formaldehyde. At pH 4, some additional 2-cyanopyridine was formed and at higher pH, new compounds appeared as shown in the chromatogram depicted in Fig. 4. The appearance of 2-pyridone indicated intermediary nitrile formation. 2-Pyridine carbaldoxime was stable under the incubation conditions and did not form 2-pyridone.

References

1 F. Hobbinger in Handbook of Pharmacology *42*, 487 (1976).
2 H. Engelhard and W.D. Erdmann, Klin. Wochenschr. *41*, 525 (1963).
3 K. Schoene, Biochem. Pharmacol. *22*, 2997 (1973).
4 B. Boščović, Fund. Appl. Toxicol. *1*, 203 (1981).
5 W. Hauser, D. Kirsch, and N. Weger, Fund. Appl. Toxicol. *1*, 164 (1981).
6 L.A. Kepner and O.L. Wolthuis, Eur. J. Pharmacol. *48*, 377 (1978).
7 J. Lipp and T. Dola, Arch. Int. Pharmacodyn. Ther. 138 (1980).
8 K. Schoene, J. Steinhanses and H. Oldiges, Biochem. Pharmacol. *32*, 1649 (1983).
9 M. Škrinjarić and M. Kralj, Arch. Toxicol., *45*, 21 (1980).
10 J.C. Clement, Fund. Appl. Toxicol. *1*, 193 (1981).
11 P.M. Lundy and K.P. Tremblay, Eur. J. Pharmacol. *60*, 47 (1979).
12 D.M. Kirsch, W. Hauser and N. Weger, Fund. Appl. Toxicol. *1*, 169 (1981).
13 D. Kuhnen-Clausen, I. Hagedorn, G. Gross, H. Bayer and F. Hucho, Arch. Toxicol. *54*, 171 (1983).
14 K. Schoene, Monogr. in Neural Sciences *7*, 85, Karger, Basel 1980.
15 J.G. Clement and P.A. Lockwood, Toxicol. Appl. Pharmacol. *64*, 140 (1982).
16 M.C. French, J.R. Wetherell and Ph.D.T. White, Eur. J. Pharmacol. *91*, 399 (1983).
17 I. Christenson, Acta Pharm. Suec. *5*, 249 (1968).
18 I. Christenson, Acta Pharm. Suec. *9*, 351 (1972).
19 I. Christenson, Acta Pharm. Suec. *9*, 323 (1972).

20 N.D. Brown, L.L. Hall, H.K. Sleeman, B.P. Doctor and G.E. Demaree, J. Chromatogr. *148*, 453 (1978).
21 H.P. Benshop, A.G. Kees, K.A.G. Konings, S.P. Kossen and D.A. Ligtenstein, J. Chromatogr. *225*, 107 (1981).
22 E.C. Horning and M.G. Horning, J. Org. Chem. *11*, 95 (1946).
23 Dissertation *G. Gross*,, Freiburg 1980

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Cyclopenta[*c*]pyridine aus Baldrinal und Homobaldrinal durch Reaktion mit primären Aminen

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Baldrinal und Homobaldrinal **(1a–b)** lassen sich mit primären Aminen in Cyclopenta[*c*]pyridine **7** überführen, wobei Oxidation der Seitenkette an C-4 zum Carbaldehyd beobachtet wird. Mit biogenen Aminen wie Tyramin und Histamin entstehen entsprechend substituierte Pseudoazulene **8** und **9**.

Cyclopenta[*c*]pyridines from Baldrinal and Homobaldrinal by Reaction with Primary Amines

Baldrinal and Homobaldrinal **(1a–b)** are readily converted into cyclopenta[*c*]pyridines **7** by treatment with primary amines. In addition, oxidation of the side chain at C-4 is observed yielding the carbaldehyde. Reactions with biogenic amines such as tyramine and histamine lead to the corresponding pseudoazulenes **8** and **9**.

Aufgrund struktureller Ähnlichkeit des Baldrinals **(1a)**[1] mit dem farbigen, pflanzlichen Antibiotikum Fulvoplumierin **(2)**[2,3] vermutete man antibakterielle Wirksamkeit auch für **1**, die jedoch nur sehr schwach ausgeprägt ist. Durch Überführung von **1** in unterschiedlich substituierte Hydrazone bzw. Semicarbazone konnte eine wesentliche Wirkungssteigerung nicht erreicht werden[4]. Uns interessierte in diesem Zusammenhang der O/N-Austausch im heterocyclischen Sechsring von **1**, also die Umwandlung des Cyclopenta[*c*]pyran- in das entsprechende Cyclopenta[*c*]pyridinsystem[5]. Als Vorbild dazu diente die Biogenese der aus Valeriana officinalis L. isolierten monoterpenoiden Pyridinalkaloide **3** und **4**[6–8], die aus Iridoidvorstufen durch formalen Einbau von Ammoniak oder eines biogenen Amins entstehen sollen[9]. Wir berichten hier über Umsetzungen von Baldrinal **(1a)** oder Homobaldrinal **(1b)** mit primären aliphatischen sowie mit einigen biogenen Aminen.

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