Methoxy- and Acetoxy-8-oxoberbines – Synthesis, Antitumor Activity, and Interaction with DNA*)

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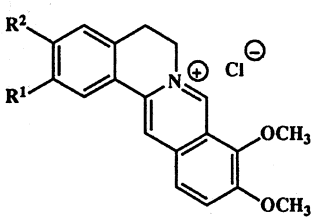
Most of the methoxy-8*H*-dibenzo[*a*,*g*]isoquinolin-8-ones **3a-h** and their acetoxy derivatives **6a-e** were synthesized by condensation of 1-oxo-1,2,3,4-tetrahydroisoquinolines **4a-c** and homophthalic acid anhydrides **5a** and **b**, ether cleavage and acetylation. These protoberberinones were tested for cytostatic activity *in vitro* using MDA-MB-231 mammary tumor cells and for interaction with native calf thymus DNA. Tetramethoxy-8-oxoberbine **3f** shows an inhibition of cell proliferation of 87% at a concentration of 10^{-5} mole; its cytostatic effect does not depend on intercalation into DNA.

Synthese, Antitumoraktivität und DNA-Interaktion von Methoxy- und Acetoxy-8-oxoberbinen

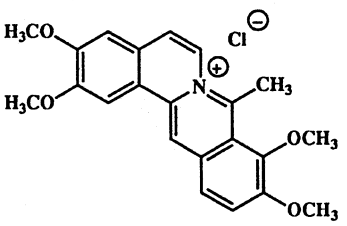
Die meisten der Methoxy-8*H*-dibenzo[*a*,*g*]chinolizin-8-one **3a-h** und ihre Acetoxyderivate **6a-e** wurden durch Kondensation der 1-Oxo-1,2,3,4-tetrahydroisochinoline **4a-c** mit den Homophthalsäureanhydriden **5a** und **b**, anschließende Etherspaltung und Acetylierung dargestellt. Die cytostatische Wirkung dieser Protoberberinone wurde an der MDA-MB-231-Zellinie, die DNA-Interaktion an nativer Kalbsthymus-DNA geprüft. Das Tetramethoxy-8-oxoberbin **3f** zeigt eine Hemmung der Zellproliferation von 87% bei einer Konzentration von 10^{-5} M, wobei die cytostatische Wirkung nicht auf eine Interkalation mit der DNA zurückzuführen ist.

In 1972 *Zee-Cheng*[1] reported upon inhibitory effects of the alkaloid berberine (**1**, Fig. 1) in various models of leukaemia. This protoberberine derivative is nearly planar and characterized by a cationic center. These two properties might contribute to the affinity of berberine (**1**) to DNA and to its cytotoxic effect[2]. Berberine (**1**) and coralyne (**2**, Fig. 1)[3], however, proved to be too toxic for clinical use and so further development of this type of alkaloids is necessary.

Based on these findings, we describe the synthesis of methoxy- (type **3**) and acetoxy-8-oxo-5,6-dihydro-dibenzo[*a*,*g*]quinolizines (type **6**), their cytotoxic properties and their interaction with DNA.



$R^1 + R^2 = -OCH_2O-$ Berberine chloride (**1**)
$R^1 = R^2 = -OCH_3$ Palmatine chloride (**14**)



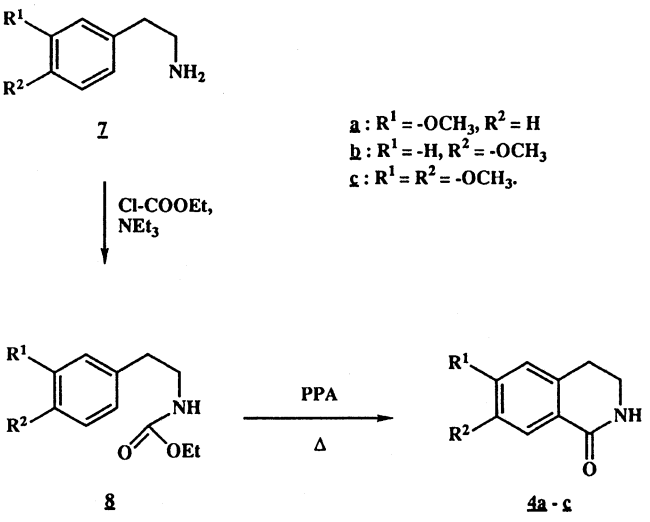
Coralyne chloride (**2**)

Figure 1

Chemistry

The demand of a large variety of dihydro-dibenzo[*a*,*g*]quinolizin-8-ones (**3**) excludes many synthetic approaches[4,5] and led us to *Haimova's* strategy[6] used for the preparation of *e.g.* 2,3,10,11-tetramethoxy-5,6-dihydro-8*H*-dibenzo[*a*,*g*]-quinolizin-8-one (**3f**), because this approach enabled us to react various 1,2,3,4-tetrahydroisoquinoline-1-ones **4** with differently substituted homophthalic acid anhydrides **5** (Scheme 1, see Page 510).

3-Methoxyphenylacetonitrile was hydrogenated with H_2/Raney-Ni. Increased pressure (60 atm) and elevated temp. (120°C) are mandatory for quantitative yields of amine **7a**. β-Phenylethylamines **7a-c** were cyclized *via* the pertinent carbamates **8a-c**[7], obtained from amines **7a-c** and ethyl chloroformate. Heating the urethanes **8a-c** with neat polyphosphoric acid affords the tetrahydroisoquinolin-1-ones **4** (Scheme 2).



Scheme 2

* Dedicated to Prof. Dr. *Hartke*, Marburg, on the occasion of his 60th birthday.

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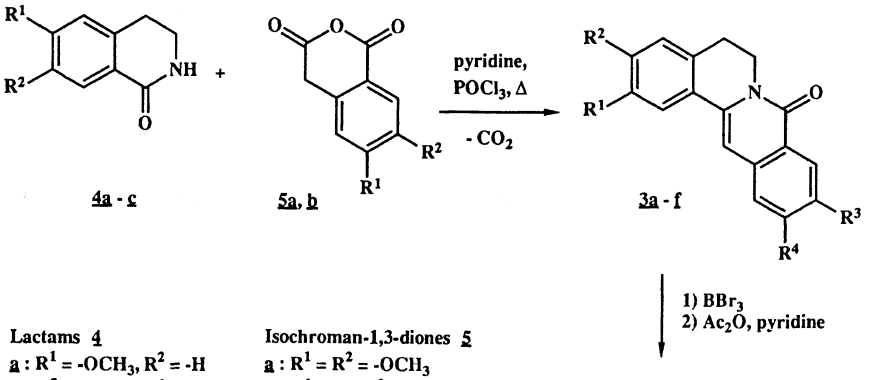


4a - c

5a, b

3a - f

6a - c
same skeleton as 3

Lactams 4
a : R^1 = -OCH_3, R^2 = -H
b : R^2 = -OCH_3, R^1 = -H
c : R^1 = R^2 = -OCH_3

Isochroman-1,3-diones 5
a : R^1 = R^2 = -OCH_3
b : R^1 = -H, R^2 = -OCH_3

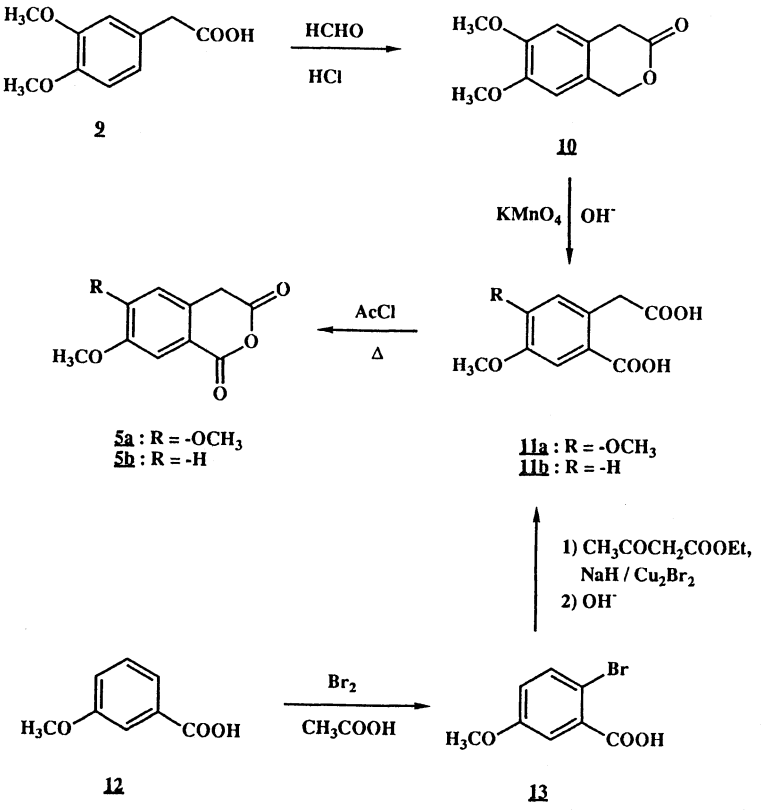
Compound	R^1	R^2	R^3	R^4
3a	OCH_3	H	OCH_3	H
3b	H	OCH_3	OCH_3	H
3c	OCH_3	OCH_3	H	H
3d	H	OCH_3	OCH_3	OCH_3
3e	OCH_3	OCH_3	OCH_3	H
3f	OCH_3	OCH_3	OCH_3	OCH_3

Compound	R^1	R^2	R^3	R^4
6a	OAc	H	OAc	H
6b	H	OAc	OAc	H
6c	H	OAc	OAc	OAc
6d	OAc	OAc	OAc	H
6e	OAc	OAc	OAc	OAc

Scheme 1

Starting from phenylacetic acid **9**, the missing *o*-CH_2-O-increment was introduced according to *Finkelstein* and *Brossi* [8]. This *o*-hydroxymethylation led to the isochroman **10**, which was oxidized to the homophthalic acid **11a**. Heating of compounds **11a** and **b** with acetyl chloride led to anhydrides **5** (Scheme 3).

Deviating from this approach 5-methoxyhomophthalic acid (**11b**) was obtained from bromination of *m*-methoxy-



9

10

5a : R = -OCH_3
5b : R = -H

11a : R = -OCH_3
11b : R = -H

12

13

Scheme 3

benzoic acid (**12**) affording **13**, and reaction of **13** with ethyl acetoacetate/NaH/Cu_2Br_2 as described as a general synthesis of homophthalic acids by *Bruggink*[9]. As outlined in the introduction *Haimova's* approach[6] was used for the synthesis of compounds **3a-f** (Scheme 1).

For reasons of comparison we also synthesized the acetoxy derivatives **6a-e**. By using BBr_3 for the cleavage of the methoxy ethers **3** we found a smooth way for getting the hydroxy derivatives - the temp. used in this procedure is depending on the grade of substitution in the aromatic rings. To avoid oxidative degradation of the free phenols we immediately prepared the acetoxy-8-oxo-5,6-dihydro-dibenzo[*a*,*g*]quinolizines **6** by refluxing with acetic acid anhydride/pyridine (Scheme 1).

To get the 9,10-dimethoxy-8-oxo-5,6-dihydro-dibenzo[*a*,*g*]quinolizines **3g** and **h** (Scheme 4) an easy one-step synthesis is the alkaline oxidation of quaternary protoberbinium salts, already described by *Gadamer*[10], starting from berberine- (**1**) and palmatine chloride (**14**).

For further comparison of the cytotoxic properties and structure-activity-relationships we also prepared the enamine **15** as a crystalline product[11] by reduction of compound **3f** with $LiAlH_4$ (Scheme 5). Further reduction of **15** with $NaBH_4$ led to racemic xylopinine (**16**) which was dehydrogenated with iodine to the quaternary 2,3,10,11-tetramethoxyberbinium iodide **17** (Scheme 5).

The NMR-Data of the intermediate 2,3,10,11-tetramethoxyenamine **15** were assigned by NOE-difference-spectra.

Because 5,6-dihydro-8-oxoberbines have a slight angle (18-20°, Fig. 2) in the isoquinoline ring system and the cytotoxic properties often depend on the planarity of a substance, we aromatized the 5,6-dihydro-8-oxoberbine **3f** by dry heating with Pd/C. According to *Dreiding*-models and the rules of aromaticity the resulting 8*H*-dibenzo[*a*,*g*]quinolizin-8-one **18** (Scheme 4) has a planar ring system with 18 π-electrons.

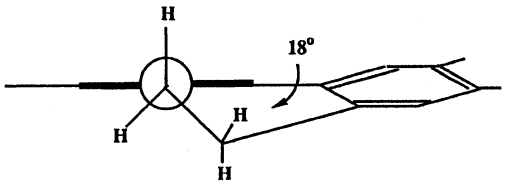
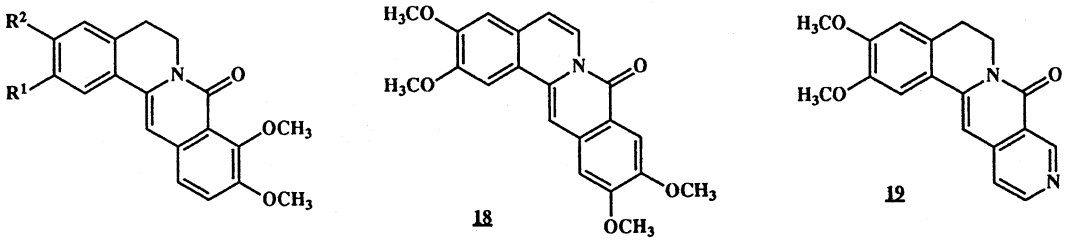
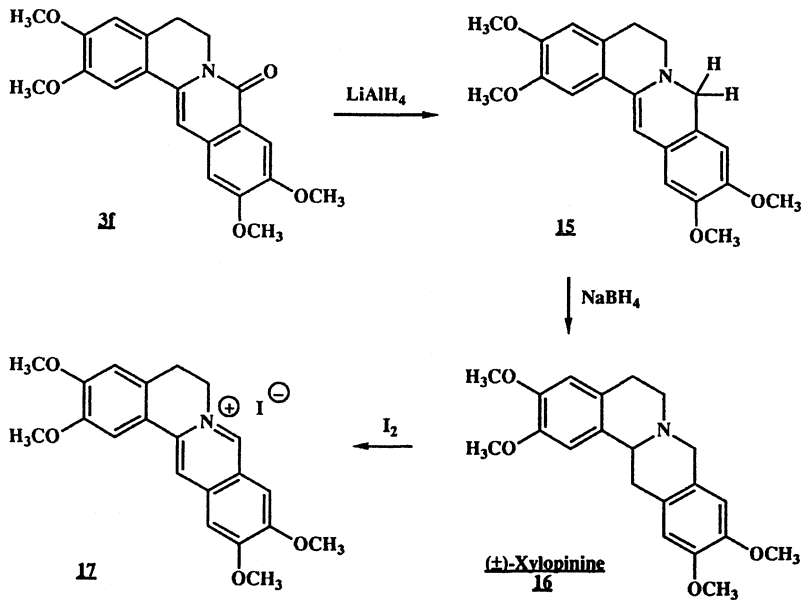


Fig.2: Deviation from planarity (see text).



Scheme 4



Scheme 5

Cytostatic activity

The MDA-MB-231 cell line[12] was used for determination of the cytostatic activity. The cells are hormone-independent mammary tumor cells of human origin. All the 8-oxoberbines described were tested for cytostatic effects at a conc. of 10^{-5} mole. The inhibition of cell growth was determined by the microtiter essay with spectroscopic measurement of the living cell density[13].

Most of our compounds were devoid of activity, with the exception of **3d** and **3f**, which had equal or better cytostatic properties than berberine (**1**) and coralyne chloride (**2**). The tetramethoxy-8-oxoberbine **3f** was the most active compound. Replacement of the methoxyphenyl ring (ring D) by a pyridine ring (**19**[14], Scheme 4) decreased the cytostatic activity.

Table 1: Cytostatic effect on the growth of MDA-MB 231 cells

Compound	% T/C [a]	Compound	% T/C [a]
1	41 [b]	6a	99 [ns]
2	33 [b]	6b	76 [b]
3a	95 [ns]	6c	77 [b]
3b	99 [ns]	6d	56 [b]
3c	70 [b]	6e	69 [b]
3d	44 [b]	16	75 [b]
3e	98 [ns]	17	100 [ns]
3f	13 [b]	18	100 [ns]
3g	61 [b]	19	89 [b]
3h	81 [b]	23	97 [ns]

[a] Concentration 1×10^{-5} M.
[b] significant $p < 0.01$.
[ns] not significant.

Interaction with DNA/RNA

Interaction of a compound with DNA or RNA often leads to cytostatic effects. In this context intercalation has to be distinguished from unspecific addition to DNA or RNA.

Conventional UV-spectroscopy is frequently used for preliminary studies of interactions with DNA. Berberine (**1**), e.g., when incubated with native DNA, shows three isosbestic points in its UV-spectrum, which according to *Krey*[2] might be indicative for a certain affinity of **1** to DNA. Coralyne (**2**), however, does not exhibit an isosbestic point under these conditions, but a hypsochromic effect at low concentrations was regarded[15] as a hint towards an interaction with DNA; high concentration of **2** leads to a bathochromic shift[2].

In UV-difference spectroscopy even small deviations from the spectrum taken without DNA can be recognized by shifts of the base line. Sufficient solubility in the DNA-buffer system is a prerequisite for pertinent investigations. We tested our dibenzo[*a*,*g*]quinolizin-8-ones in comparison with ethidium bromide and berberine chloride (**1**): Most of our protoberberines, especially di- and trimethoxy-derivatives did not show any or only weak interactions with native DNA. Tetramethoxy-substitution, however, causes interference (Fig. 3).

As expected ethidium bromide shows strongest interaction efficacy, followed by compound **3f** (line 2). Berberine chloride (**1**) was nearly ineffective under these conditions. In order to find out whether the interaction of compound **3f** with DNA depends on its concentration, constant aliquots of **3f** were added to the DNA-solution: linear increase points towards a concentration-depending interaction with native DNA. After sonication of native DNA ethidium bromide has lost its affinity to DNA whilst the difference spectra with **3f** do not differ from each other. Therefore, we suppose that this protoberberinone affects not only the intact DNA but also single DNA-increments. As a consequence we tested interferences of **3f** with guanosine, cytidine, adenosine, uridine, and their 5-phosphates as well as with pertinent 2'-desoxy derivatives and thymidine: **3f** shows affinity to 2'-desoxyguanosine, 2'-desoxycytidine and to their phosphates (Fig. 4).

The interaction with 2'-desoxyadenosine is weak, no effect was observed with 2'-desoxyuridine and thymidine. Therefore, we do not expect any specific affinity to native RNA. The cytotoxic trimethoxyprotoberberine **3d** does not show any interaction with native DNA or its nucleosides.

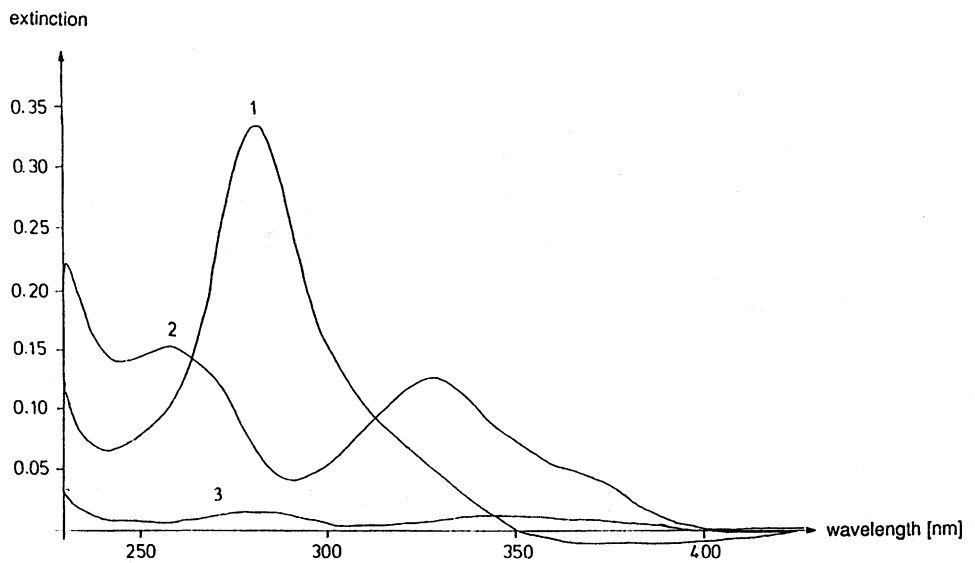


Fig. 3: Difference spectra for 1 = **ethidium bromide**, 2 = **3f**, and 3 = **berberine chloride**; R = [substance]/[DNA] = 1.5 [mole/mole phosphorus].

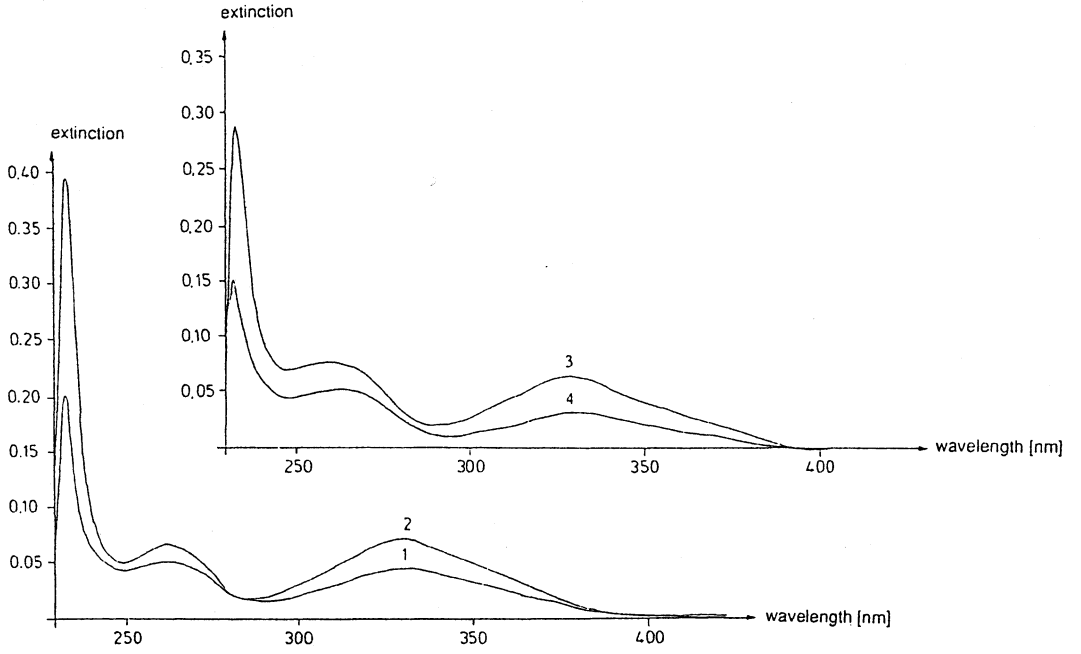


Fig. 4: Difference spectra for **3f** with 1 = 2'-desoxyguanosine, 2 = 2'-desoxyguanosine phosphate, 3 = 2'-desoxycytidine and 4 = 2'-desoxycytidine phosphate.

Fluorescence Spectroscopy

The interaction of **3f** with DNA was confirmed by DNA-induced shift of its fluorescence maximum from 425 nm (**3f** in DMF plus phosphate buffer saline) to 540 nm after incubation with DNA for 2 min (cf. Experimental Part).

Displacement of Ethidium bromide

At high concentrations of salts in the buffer system (ca. 1.0 mole) ethidium bromide interacts with DNA by intercalation, whilst at low ionic strength additional electrostatic binding occurs, caused by reciprocal actions of the phosphate increments with the ethidium cation. These two effects lead to an increase of fluorescence of the ethidium cation which is considered to be a specific test for intercalation[16,17]. Addition of another intercalating substance displaces ethidium bromide and, therefore, diminishes or cancels its fluorescence intensity, depending on the concentration of the displacing compound. The C_{50}-values (50% fluorescence quenching) obtained according to *McGhee* and *von Hippel*[18] are used to determine binding constants of our substances under consideration.

For our experiments we have used the ratio [DNA = mole phosphorus]/[mole ethidium bromide] = 2.0 in order to get sufficient fluorescence intensity at the beginning of the titration. Moreover, a low ionic strength was adjusted to leave both opportunities of binding open (*vide supra*). Experiments were run with those protoberberinones exhibiting significant effects at MDA-MB-231 cells (Table 1)[19]. Actinomycin D was used as a positive control[20]; this compound displaces ethidium bromide irreversibly. Fig. 5 shows that actinomycin D replaces ethidium bromide, whilst the protoberberinones tested do not.

Viscosity experiments

Intercalating substances lead to increased length of DNA for about 3.4 Å by partial unwinding[21] accompanied by changing of the torsion angle (ethidium bromide, e.g., gives rise to an alteration of a least 12° [22]). At the same time intercalation stabilizes DNA so increasing its viscosity. Therefore, intercalating properties can be determined by titration of DNA with the intercalating molecule. By this method intercalation of coralyne (**2**) into native DNA - analogous to that of ethidium bromide - has been detected[15].

Titration of native DNA with protoberberinone **3f** indicated that this test compound - contrary to ethidium bromide - does not increase the relative viscosity of DNA, so corroborating the results of the displacement experiment and of the test with sonicated DNA. Obviously **3f** (and similar protoberberinones?) does interfere with native DNA, but not by intercalation.

Discussion

Among the tetramethoxy-8*H*-dibenzo[*a*,*g*]quinolizin-8-ones, only **3f** shows strong cytostatic activity against MDA-MB-231 human mammary tumor cells with a better effect than the original protoberberines berberine (**1**) or coralyne chloride (**2**). This effect can be rationalized by an interaction with DNA. However, a direct intercalation into DNA can be ruled out. Based on these findings, we have synthesized C-5-, C-6- and C-13-alkylated derivatives to improve these effects[19]. In the case of the acetoxy derivatives, this structural modifications should give rise to binding affinity for the estrogen receptor, a prerequisite for a selective action on estrogen receptor positive tumors like mammary carcinomas[19].

The authors thank *M. Beer* and *R. Brunner-Ploss* for technical assistance and the Deutsche Forschungsgemeinschaft (SFB 234) for financial support.

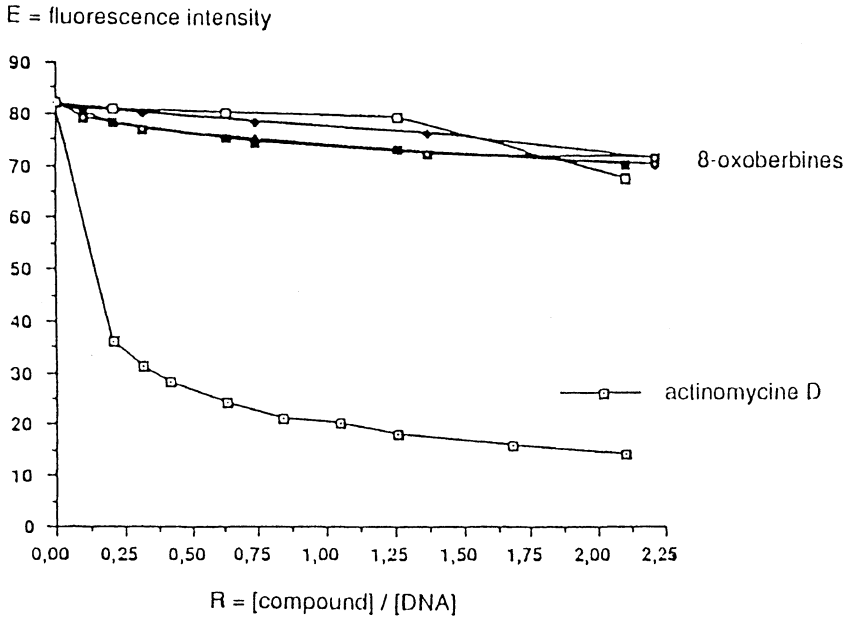


Fig. 5: Fluorescence intensity, plotted against the molar relation [compound]/[DNA].

Experimental Part

Melting points: Büchi 510 apparatus, uncorrected.- Elemental analyses: Mikroanalytisches Laboratorium, University of Regensburg.- IR-spectra: Beckman Acculab III; KBr.- ^{1}H-NMR-spectra: Varian EM 390 (90 MHz), Bruker WM 250 (250 MHz); TMS as internal standard.- UV-(difference)spectra: Uvikon 810 (Kontron); solvent: acetonitrile.- Fluorescence-spectra: Hitachi F-3000.- Mass-spectra: Varian MAT CH 5.

Dihydro-dibenzo[a,g]quinolizines **3a-f**

1-Oxoisoquinoline **4** (5 mmole) is suspended in chlorobenzene p.a. (15 ml), pyridine p.a. (0.41 ml) and $POCl_3$ (0.21 ml in 5 ml chlorobenzene p.a.) are added. The suspension is stirred for 15 min at room temp., then homophthalic acid anhydride **5** (4.55 mmole) is added slowly. The mixture is stirred again for 15 min, then refluxed for 1 h, cooled again and dissolved in CH_2Cl_2 (50 ml). The org. layer is washed with 10% NaOH (3 x 40 ml), dried (Na_2SO_4) and evaporated. The remaining oil is purified by CC (SiO_2; EtOAc) and crystallized from ether. The crystals of **3a-f** are colorless and recrystallized from EtOH.- Yields: 55-70%.

5,6-Dihydro-2,10-dimethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3a**)

3a was synthesized from **4b** and **5b**; m.p. 152°C.- $C_{19}H_{17}NO_3$ (307.4) Calc. C 74.3 H 5.58 N 4.6 Found C 74.0 H 5.55 N 4.4.- IR (KBr): 1645 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.88 (t; J = 6 Hz; 2H; -CH_2-; H-5), 3.80 (s; 3H; -OCH_3), 3.87 (s; 3H; -OCH_3), 4.33 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.73-7.90 (m; 6H arom and 1H vinyl).- UV: λ max (log ε) = 326 (4.27), 255 (4.12), 221 nm (4.26).

5,6-Dihydro-3,10-dimethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3b**)

From **4a** and **5b**; m.p. 176-177°C.- $C_{19}H_{17}NO_3 \cdot 1/2\ H_2O$ (316.4) Calc. C 72.2 H 5.73 N 4.4 Found C 72.0 H 5.81 N 4.3.- IR (KBr): 1645 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.87 (t; J = 6 Hz; 2H; -CH_2-; H-5), 3.77 (s; 3H; -OCH_3), 3.87 (s; 3H; -OCH_3), 4.33 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.57-7.92 (m; 6H arom and 1H vinyl).- UV: λ max (log ε) = 327 (4.38), 255 (4.17), 214 nm (4.44).- MS: m/z = 307 (100%, M^+), 292 (80, * 277.73 (M - CH_3)$^+$), 277 (4), 153.5 (10, M^{2+}).

5,6-Dihydro-2,3-dimethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3c**)

From **4c** and homophthalic acid anhydride (Fa. Aldrich); m.p. 175°C, lit. 181-182°C[23].

5,6-Dihydro-3,10,11-trimethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3d**)

From **4a** and **5a**; m.p. 185-186°C.- $C_{20}H_{19}NO_4$ x 1/2 EtOH (360.4) Calc. C 70.0 H 6.15 N 3.9 Found C 70.3 H 5.82 N 4.1.- IR (KBr): 1650 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.93 (t; J = 6 Hz; 2H; -CH_2-; H-5), 3.82 (s; 3H; -OCH_3), 3.97 (s; 6H; -OCH_3), 4.35 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.70-7.03 (m; 3H arom and 1H vinyl), 7.60-7.90 (m; 2H arom).- UV: λ max (log ε) = 328 (4.27), 252 (4.31), 218 nm (4.29).

5,6-Dihydro-2,3,10-trimethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3e**)

From **4c** and **5b**; m.p. 157-158°C.- $C_{20}H_{19}NO_4$ (337.4) Calc. C 71.2 H 5.68 N 4.2 Found C 70.8 H 5.60 N 4.0.- IR (KBr): 1645 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.87 (t; J = 6 Hz; 2H; -CH_2-; H-5), 3.85 (s; 6H; -OCH_3), 3.93 (s; 3H; -OCH_3), 4.32 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.65, 6.77 (s; 1H arom and 1H vinyl), 7.07-7.53 (m; 3H arom), 7.77 (d; J = 2 Hz; 1H arom; H-9).- UV: λ max (log ε) = 322 (4.31), 256 (4.04), 217 nm (4.25).

5,6-Dihydro-2,3,10,11-tetramethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3f**)

From **4c** and **5a**; m.p. 194°C, lit. 190°C[24].- MS: m/z = 367 (100%, M^+), 352 (54, * 337.61 (M - CH_3)$^+$), 183.5 (23, M^{2+}).

Lactams **4a-c**

Polyphosphoric acid (100 g) is preheated to 140°C (oil bath temp.). After reaching this temp. carbamate **8** (50 mmole) is directly poured onto the polyphosphoric acid and refluxed for 1 h. After cooling the mixture is suspended in ice water (100 ml). While cooling the solution is alkalized with NaOH (40%) and extracted with CH_2Cl_2 (6 x 150 ml). The combined org. layers are dried (Na_2SO_4) and evaporated. The remaining oil is purified by CC (SiO_2; EtOAc) and crystallized from ether. The crystals of **4a-c** are colorless and recrystallized from ether.- Yields: 50-60%.

1,2,3,4-Tetrahydro-6-methoxy-1-oxo-isoquinoline (**4a**)

Prepared from **8a**; m.p. 139°C, lit. 139°C[25].- UV: λ max (log ε) = 285 (3.35), 250 nm (4.09).

1,2,3,4-Tetrahydro-7-methoxy-1-oxo-isoquinoline (**4b**)

From **8b**; m.p. 86°C, lit. 86-88°C[26].- UV: λ max (log ε) = 299 (3.37), 229 nm (3.96).

1,2,3,4-Tetrahydro-6,7-dimethoxy-1-oxo-isoquinoline (**4c**)

From **8c**; m.p. 174°C, lit. 173.5-174.5°C[27].- UV: λ max (log ε) = 293 (3.76), 255 nm (3.94).

Carbamates **8a-c**

100 mmole of the corresponding β-phenylethylamine **7** are dissolved with NEt_3 (10.0 g) in absol. CH_2Cl_2 (50 ml). After addition of ethyl chloroformate (10.8 g) in 50 ml absol. CH_2Cl_2 the mixture is stirred for 1 h. The precipitate is dissolved with 2 N HCl and stirred again. After washing with H_2O the org. layer is dried (Na_2SO_4) and evaporated. The remaining oils or precipitates are used without further purification.- Yields: nearly quantitative.

Ethyl-N-[2-(3-methoxyphenyl)ethyl]-carbamate (**8a**)

Prepared from **7a** and ethyl chloroformate (Fa. Aldrich); $C_{12}H_{17}NO_3$ (223.3).- IR (film): 3340 (-NH-), 1710 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.25 (t; J = 7.5 Hz; 3H; $-CH_3$), 2.80 (t; J = 7.5 Hz; 2H; $-C\underline{H}_2-CH_2$-NH-), 3.40 ("q"; J = 7.5 Hz; 2H; $-CH_2-C\underline{H}_2$-NH-), 3.77 (s; 3H; $-OCH_3$), 4.10 (q; J = 7.5 Hz; 2H; $-C\underline{H}_2-CH_3$), 4.90 (s broad; 1H; -NH-; not exchangeable), 6.67-7.40 (m; 4H arom).

Ethyl-N-[2-(4-methoxyphenyl)ethyl]-carbamate (**8b**)

From β-(p-methoxyphenyl)ethylamine and ethyl chloroformate; colorless crystals, m.p. 45°C (ether).- $C_{12}H_{17}NO_3$ (223.3) Calc. C 64.6 H 7.67 N 6.3 Found C 64.4 H 7.64 N 6.2.- IR (KBr): 3340 (-NH-), 1710 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.20 (t; J = 7.5 Hz; 3H; $-CH_2-C\underline{H}_3$), 2.70 (t; J = 7.5 Hz; 2H; $-C\underline{H}_2-CH_2$-NH-), 3.40 ("q"; J = 7.5 Hz; 2H; $-CH_2-C\underline{H}_2$-NH-), 3.77 (s; 3H; $-OCH_3$), 4.10 (q; J = 7.5 Hz; 2H; $-C\underline{H}_2-CH_3$), 4.67 (s broad; 1H; -NH-; not exchangeable), 6.70-7.33 (m; 4H arom).

Ethyl-N-[2-(3,4-dimethoxyphenyl)ethyl]-carbamate (**8c**)

From homoveratrylamine (Fa. Fluka) and ethyl chloroformate; white solid, m.p. 61-62°C (ether), lit. 64-66°C[28].

1-Amino-2-(3-methoxyphenyl)ethane (**7a**)

200 mmole 3-methoxyphenylacetonitrile (Fa. Fluka), dissolved in EtOH (150 ml, saturated with NH_3) are hydrogenated in the presence of Raney-Ni (from 1.5 g alloy) at 60 atm and 120°C for about 4 h. The catalyst is filtered off, washed with EtOH and the solvent is evaporated: pure, colorless oil, b.p. 87°C, 0.2 T; lit. 122-123°C, 7 mm[29].- Yield: 90-95%.

Isochroman-1,3-diones **5a** *and* **b**

A suspension of homophthalic acid **11** (15 mmole) and acetylchloride (30 ml) are refluxed under stirring for 1 h, then the excess of reagent is distilled off. 25 ml of absol. ether are added to the residue, the crystallized product is washed with ether and dried.- Yields: 60-65%.

6,7-Dimethoxy-isochroman-1,3-dione (**5a**)

Prepared from **11a**; colorless crystals, m.p. 174-176°C (ether), lit. 175°C[30].

7-Methoxy-isochroman-1,3-dione (**5b**)

From **11b**; light-brown crystals, m.p. 131-134°C (ether), lit. 142-143°C[31].

6,7-Dimethoxyisochroman-3-one (**10**)

3,4-Dimethoxyphenylacetic acid (**9**) (200 mmole; Fa. Merck) is heated with glacial acetic acid (200 ml) on the steam bath. Formaline solution (37%, 40 ml) and conc. HCl (40 ml) are added and the mixture is stirred on the steam bath under reflux for 1 h. H_2O (200 ml) is added, the solution is extracted with $CHCl_3$ (3 x 200 ml). The combined org. layers are washed with 10% $NaHCO_3$-solution, dried (Na_2SO_4) and evaporated. The residue is crystallized by adding some drops of EtOH or ether.- Yield: 90%.- Colorless crystals, m.p. 98-99°C (EtOH), lit. 102-103°C[32].

4,5-Dimethoxyhomophthalic acid (**11a**)

100 mmole lactone **10** are dissolved in 10% KOH/H_2O (110 ml). Under stirring 2% $KMnO_4$-solution (1600 ml) is added quickly, the solution is stirred at room temp. for 10 h. EtOH (20 ml) is added and the mixture is heated on the steam bath. After filtration the water layer is concentrated and acidified with conc. HCl. The precipitate is recrystallized from H_2O.- Yield: 90-95%.- Colorless crystals, m.p. 206-208°C (H_2O), lit. 213-214°C[32].

2-Bromo-5-methoxybenzoic acid (**13**)

0.24 mole of m-anisic acid (Fa. Aldrich) are dissolved in NaOH/H_2O (10.7 g/267.5 mmole NaOH in 400 ml H_2O). After heating to 50-60°C 12.9 ml (0.25 mole) of Br_2 are added slowly, then the solution is stirred for 30 min. While cooling $NaHSO_3$-solution is added until the mixture is colorless. The precipitate is dried.- Yield: 70-80%.- Colorless crystals, m.p. 157-158°C (EtOH 96%), lit. 162°C[31].

5-Methoxyhomophthalic acid (**11b**)

11b is prepared from **13** according to *Bruggink*[9].- Yield: 60-70%; light-brown crystals, m.p. 184-186°C (CH_2Cl_2/MeOH/H_2O), lit. 184-186°C[9].

Acetoxy-dihydro-dibenzo[a,g]quinolizines **6a-e**
a) Ether cleavage

100 mg methoxyoxoberbine **3** (in 10 ml of absol. CH_2Cl_2) are added dropwise to 0.1 ml of BBr_3 in 10 ml of absol. CH_2Cl_2, then refluxed for 1 h and stirred at room temp. for 12 h. While cooling saturated $NaHCO_3$-solution (10-15 ml) is added and the suspension is mixed with EtOAc to get a clear solution. The org. layer is separated, the water layer is extracted again with EtOAc (2 x 50 ml). The combined org. layer are washed with saturated NaCl-solution, dried (Na_2SO_4) and evaporated. Generally the residue is dried for a short time i. vac., then it is acetylated immediately.

b) Acetylation

The residue from a) is refluxed with Ac_2O (6 ml) and absol. pyridine (5 drops) for 2 h, then the excess of reagents is evaporated i. vac. After cooling the remaining oil is crystallized from MeOH and the precipitate is washed with ether and dried. Purification: CC (SiO_2, EtOAc).- Yields: 70-80% colorless crystals.

2,10-Diacetoxy-5,6-dihydro-8H-dibenzo[a,g]quinolizin-8-one (**6a**)

Prepared from **3a**; m.p. 168-169°C (MeOH).- $C_{21}H_{17}NO_5 \cdot 1/2\ CH_3OH$ (379.4) Calc. C 68.1 H 5.05 N 3.7 Found C 68.4 H 4.90 N 3.8.- IR (KBr): 1750 (CH_3-CO), 1660 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.30 (s; 6H; H_3C-CO-), 2.97 (t; J = 6 Hz; 2H; $-CH_2$-; H-5), 4.35 (t; J = 6 Hz; 2H; $-CH_2$-; H-6), 6.87-7.63 (m; 5H arom and 1H vinyl), 8.10 (d; J = 2 Hz; 1H arom; H-9).- UV: λ max (log ε) = 329 (4.28), 212 nm (4.40).

3,10-Diacetoxy-5,6-dihydro-8H-dibenzo[a,g]quinolizin-8-one (**6b**)

From **3b**; m.p. 206-208°C (MeOH).- $C_{21}H_{17}NO_5$ (363.4) Calc. C 69.4 H 4.72 N 3.9 Found C 69.1 H 4.83 N 3.9.- IR (KBr): 1750 (CH_3-CO), 1640 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.33 (s; 6H; H_3C-CO-), 3.00 (t; J = 6 Hz; 2H; -CH_2-; H-5), 4.42 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.90-8.20 (m; 6H arom and 1H vinyl).- UV: λ max (log e) = 331 (4.34), 209 nm (4.79).

3,10,11-Triacetoxy-5,6-dihydro-8H-dibenzo[a,g]quinolizin-8-one (**6c**)

From **3d**; m.p. 217-219°C (MeOH).- $C_{23}H_{19}NO_7$ · 1/2 CH_3OH (437.4) Calc. C 64.5 H 4.84 N 3.2 Found C 64.7 H 4.73 N 3.0.- IR (KBr): 1775 (CH_3-CO), 1645 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.32 (s; 9H; H_3C-CO-), 2.93 (t; J = 6 Hz; 2H; -CH_2-; H-5), 4.32 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.92 (d; J = 2 Hz; 1H arom; H-4), 7.00-7.12 (m; 1H arom and 1H vinyl), 7.42 (s; 1H arom), 7.78 (dd; $J_{1/2}$ = 9/2 Hz; 1H arom; H-2), 8.22 (s; 1H arom).- UV: λ max (log ε) = 326 (4.32), 216 nm (4.33).

2,3,10-Triacetoxy-5,6-dihydro-8H-dibenzo[a,g]quinolizin-8-one (**6d**)

From **3e**; m.p. 202-205°C (MeOH).- $C_{23}H_{19}NO_7$ · 1/2 H_2O (430.4) Calc. C 64.1 H 4.68 N 3.3 Found C 63.8 H 4.66 N 3.0.- IR (KBr): 1765; 1775 (CH_3-CO), 1660 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.17 (s; 3H; H_3C-CO-), 2.30 (s; 6H; H_3C-CO-), 2.97 (t; J = 6 Hz; 2H; -CH_2-; H-5), 4.35 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.93 (s broad; 1H vinyl; H-13), 7.13 (s broad; 1H arom), 7.43 ("d"; J = 2 Hz; 1H arom), 7.53 (s; 1H arom), 7.63 (s; 1H arom), 8.08 (d; J = 2 Hz; 1H arom; H-9).- UV: λ max (log ε) = 330 (4.30), 215 nm (4.37).

2,3,10,11-Tetraacetoxy-5,6-dihydro-8H-dibenzo[a,g]quinolizin-8-one(**6e**)

From **3f**; m.p. 244-245°C (MeOH).- $C_{25}H_{21}NO_9$ (479.4) Calc. C 62.6 H 4.41 N 2.9 Found C 62.2 H 4.04 N 2.8.- IR (KBr): 1775 (CH_3-CO), 1655 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.30 (s; 12H; H_3C-CO-), 2.96 (t; J = 6 Hz; 2H; -CH_2-; H-5), 4.33 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.87, 7.12, 7.41, 7.61, 8.20 (s; 4H arom and 1H vinyl).- UV: λ max (log ε) = 329 (4.34), 216 nm (4.37).

Dihydro-dibenzo[a,g]quinolizines **3g** *and* **h**

To 50 mmole berberin- (**1**) or palmatine chloride (**14**), dissolved in hot H_2O (500 ml), are dropped 150 g KOH in 70 ml H_2O. The mixture is stirred for 15 min and extracted with ether (3 x 500 ml). The black-brown precipitate is stirred with hot ether for 15 min and filtrated. The combined ether layers are washed with water, dried (Na_2SO_4) and evaporated. Purification: CC (SiO_2, EtOAc).- Yields: 40-50%.

5,6-Dihydro-9,10-dimethoxy-2,3-methylendioxy-8H-dibenzo[a,g]-quinolizin-8-one (**3g**)

From berberine chloride (Fa. Sigma); white-yellow crystals, m.p. 188-190°C (EtOH), lit. 199.5°C[33].

5,6-Dihydro-2,3,9,10-tetramethoxy-8H-dibenzo[a,g]quinolizin-8-one (**3h**)

From palmatine chloride (Fa. Sigma); yellow foam, m.p. 183-184°C (EtOH, lit. 183°C[34]).- $C_{21}H_{21}NO_5$ · 1/2 EtOH (390.4) Calc. C 67.7 H 6.15 N 3.6 Found C 67.7 H 5.87 N 3.5.- IR (KBr): 1655 (-CO) cm^{-1}.- ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 2.90 (t; J = 6Hz; 2H; -CH_2-; H-5), 3.88, 3.93, 3.97, 4.00 (s; 12H; -OCH_3), 4.33 (t; J = 6 Hz; 2H; -CH_2-; H-6), 6.73, 6.78 (s; 2H arom), 7.23-7.32 (m; 2H arom and 1H vinyl).- UV: λ max (log ε) = 327 (4.26), 217 nm (4.26).

5,6-Dihydro-2,3,10,11-tetramethoxy-8H-dibenzo[a,g]quinolizine(**15**)

The enamine **15** is prepared from the 8-oxoberbine **3f** according to *Kiparissides*[11]. Yield: 85-90%; yellow precipitate, $C_{21}H_{23}NO_4$ (353.4), m.p. 192-195°C (dec.), lit. 173-176°C[11].- NOE-difference-spectra (250 MHz):

a) *Enamine:* 2.90 (t; J = 6 Hz; 2H; -CH_2-; H-5), 3.13 (t; J = 6 Hz; 2H; -CH_2-; H-6), 3.87, 3.88, 3.89 (s; 9H; -OCH_3), 3.94 (s; 3H; -OCH_3, C-2 or C-11), 4.13 (s; 2H; -CH_2-; H-8), 6.03 (s; 1H vinyl; H-13), 6.60 (s; 1H arom; H-4), 6.62 (s; 1H arom; H-1 or H-12), 6.63 (s; 1H arom; H-9), 7.12 (s; 1H arom; H-1 or H-12).- UV: λ max (log ε) = 375 (4.13), 343 (4.09), 285 (4.35), 263 nm (4.19).- MS: m/z = 353 (81%, M^+), 352 (100, * 351.00 $(M - H)^+$), 338 (20, $(M - CH_3)^+$), 337 (10, * 322.64 $(352 - CH_3)^+$), 336 (21, * 335.00 $(337 - H)^+$), 322 (5), 308 (7), 279 (8), 192 (18), 176.5 (10, M^{2+}).

b) *Iminium-Salt, trifluoroacetate:* 3.19 (t; J = 7.8 Hz; 2H; -CH_2-; H-5), 3.88 (s; 3H; -OCH_3; C-10), 3.89, 3.98 (s; 6H; -OCH_3; C-2/C-11), 4.02 (s; 3H; -OCH_3; C-3), 4.14 (t; J = 7.8 Hz; 2H; -CH_2-; H-6), 4.47 (s [broad]; 2H; -CH_2-; H-13), 5.05 (t; J = 3.8 Hz; 2H; -CH_2-; H-8), 6.75 (s; 1H arom; H-4), 6.81 (s; 1H arom; H-1 or H-12), 6.87 (s; 1H arom; H-4), 7.35 (s; 1H arom; H-1 or H-12).- UV (acetonitrile plus HCl): λ max (log ε) = 341 (4.41), 287 (4.35), 239 nm (4.27).

(±)-Xylopinine (**16**)

(±)-Xylopinine (**16**) is prepared from enamine **15** according to *Kiparissides*[11].- Purification: CC (SiO_2, EtOAc).- Yield: 70-80%, yellow crystals, m.p. 161-163°C (EtOH), lit. 156-157°C[11].

2,3,10,11-Tetramethoxyberbinium iodide (**17**)

300 mg (±)-Xylopinine (**16**) (0.82 mmole) are dissolved in absol. MeOH (25 ml) under N_2. To the refluxing solution are added 0.64 g I_2 in 30 ml absol. MeOH until the suspension turned brownish. After refluxing for 30 min $NaHSO_3$-solution is added until a yellow colour remains. The yellow precipitate is washed with water and ether and dried.- Yield: 80-90%, yellow crystals, m.p. 255°C (dec., EtOH/H_2O), lit. 245°C (dec.)[35].

2,3,10,11-Tetramethoxy-8H-dibenzo[a,g]quinolizin-8-one (**18**)

300 mg 5,6-Dihydro-8-oxoberbine **3f** are mixed thoroughly with 300 mg of Pd/C (10%) under N_2 for 15 min. The mixture is heated in an oil bath (bath temp. 220°C) for 1 h. After cooling the product is separated from the catalyst by extraction with CH_2Cl_2 and filtration. The org. layer is evaporated and dried.- Purification: CC (SiO_2, EtOAc) or PTLC (SiO_2, ether).- Yield: 80-90%; yellow crystals, m.p. 192-194°C (EtOH), lit. 196-197°C[36].- IR (KBr): 1650 (-CO) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.98 (s; 3H; -OCH_3), 4.03 (s; 6H; -OCH_3), 4.08 (s; 3H; -OCH_3), 6.37 (d; J = 7.5 Hz; 1H; H-5), 6.85, 6.97, 7.17, 7.50, 7.82 (s; 4H arom and 1H vinyl), 8.35 (d; J = 7.5 Hz, 1H; H-6).- UV: λ max (log ε) = 422 (3.97), 398 (4.07), 376 (4.04), 293 (4.42), 261 (4,41), 224 nm (4.47).

10-Aza-5,6-dihydro-2,3-dimethoxy-8H-dibenzo[a,g]quinolizin-8-one(**19**)

Prepared from 3,4-dihydro-6,7-dimethoxy-1-methyl-isoquinoline[37] and nicotinic acid chloride according to *Lenz*[14].- Yield: 40%.- Yellow-orange crystals, m.p. 162-165°C (EtOH), lit. 169-172°C[14].

Biological methods

MDA-MB 231 Human Breast Cancer Cells[12]

Cells were grown in *McCoy* medium (Fa. Boehringer, Mannheim) supplemented with 10% newborn calf serum (NCS, Fa. Gibco), gentamicin (40 µg/ml) and $NaHCO_3$ (2.2 g/l, pH = 7.35). Cells were grown in a humidified incubator in 5% CO_2 at 37°C and harvested with 0.05% trypsine-0.02% EDTA in 0.15 M NaCl. At the beginning of the experiment the cell suspension is switched to 96-holes mikrotitre dishes (100 µl/hole). After 2-3 days of incubation at 37°C the medium is changed to substance-containing medium. Control holes (16/dish) are containing 0.1% of the pertinent solvent only. The starting cell density is determined by adding vinblastine in 18 holes. After further incubation (3 days) the medium is removed and gluta-

raldehyde (100 µl) in phosphated buffer saline (PBS) (1%) is added for fixation. After 15 min the aldehyde is decanted and after 25 min the cells are colored with 100 µl of a crystal violet-solution (0.02% in H_2O). After this solution is decanted the cells are washed with H_2O for a few times to remove unused staining. After addition of 100 µl EtOH the dishes are shaken for 1 h. The optical density is determined in a microtitre dish-autoreader EL 309 (Bio-tek) at 578 nm, the data are processed on an Olivetti M24-PC[13].

UV-Difference-Spectroscopy

a) Interaction with native DNA

Calfthymus-DNA type I (Fa. Sigma) is dissolved in 0.1-M-Tris-NaCl-buffer (pH 7.4) at 4°C. The concentration of the solution is about 6 x 10^{-5} M (according to phosphorus). Test substances are dissolved in DMSO (10^{-2} M solution). Tandem quartz-cells (Fa. Helma) are used. Test- and reference-cuvette are containing the DNA-solution in one part, in the other part only buffer. 1 µl of the substance-solution is added to the DNA in the test-cell and to the buffer in the reference-cell, respectively. The mixture is incubated for 2 min and after cautious stirring the extinction difference is determined between 550 and 230 nm. A solution of ethidium bromide (10^{-2} M) is used as positive control and in order to limit the volume of solvent 10 x 1 µl substance solution are added at the outmost. To determine a concentration dependence (linear increase of the plot) of the interaction between substance and DNA, the extinction is plotted versus the quotient from substance concentration and DNA-phosphorus concentration.

b) Interaction with sonicated DNA

The experiment is performed according to a). To destroy the DNA-structure the DNA-solution (see above) is sonicated for 10 min at room temp. For evaluation of the spectra identical concentrations of substances are compared (native/sonicated DNA).

c) Interaction with (non-)phosphorylated DNA-increments

The experiment is performed according to a), but a solution of (non-) phosphorylated DNA-increments (10^{-2} M) is used instead of the DNA solution.

Fluorescence Spectroscopy

To determine a DNA-induced shift in the fluorescence spectra of our substances we measured the spectra with 5 µl substance solution (10^{-2} M/DMF) in phosphated buffer saline and of 5 µl substance solution + 5 µl DNA solution (ca. 4.6 x 10^{-5} M, according to phosphorus) in PBS between 600 and 330 nm, respectively.- Excitement wavelength: 265 nm; excitement bandpass: 5 nm; emission bandpass: 5 nm; scan speed: 600 nm/min.

Displacement of Ethidium bromide

To get sufficient fluorescence intensity at the beginning of the titration we used the ratio [DNA = mole phosphorus]/[mole ethidium bromide] = 2.0. The concentration of the DNA solution (in 0.1-M-Tris-NaCl-buffer, ph 7.4) was 4.6 x 10^{-5} M (according to phosphorus), the concentration of ethidium bromide 10^{-2} M (in DMF), actinomycin D (10^{-2} M/DMF) was used as a positive control. In the experiments aliquots of the test substance solutions are added to the ethidium bromide-DNA solution, and after mixing the quenching of fluorescence intensity is determined.- Excitement wavelength: 546 nm; emission wavelength: 590 nm; excitement bandpass: 5 nm; emission bandpass: 5 nm.

Viscosity Experiments

For this experiment an *Ostwald*-viscosimeter (No. I) is used, the concentration of the DNA solution (in 0.1-M-Tris-NaCl-buffer, pH 7.4) was 4.6 x 10^{-5} M (according to phosphorus). The concentration of the test substances was 10^{-2} M (in DMF). In order to find the apparatus constant the sinking time in the buffer was determined, which was equal to that of water. The apparatus constant is calculated according to the following usual formula:

$$\gamma = \frac{\eta}{\rho} = k \cdot t$$

γ = kinematic viscosity, η = dynamic viscosity, ρ = density, k = apparatus constant, t = sinking time.

As basic value for the sinking time the DNA-solution was measured. By addition of aliquots from the substance solution to the DNA-solution in the viscosimeter the ratio between substance- and DNA concentration was raised from 0.01 to 0.1 with compensation of the solvent effects. From this experiment the corrected time for the measurements is resulting:

$$\text{Corrected time} = t_{DNA+DMF} - t_{DNA}$$

The corrected time is substracted from the sinking time of the substance solutions, the resulting value is the corrected sinking time for the substances. Now it is possible to calculate the kinematic viscosity γ:

$$\gamma = \frac{\eta}{\rho} = k \cdot t'$$

γ = kinematic viscosity, η = dynamic viscosity, ρ = density, k = apparatus constant, t' = corrected sinking time.

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