Monatshefte für Chemie 127, 97–102 (1996)

Monatshefte für Chemie
Chemical Monthly

[*a*]-Anellated Carbazoles with Antitumor Activity: Synthesis and Cytotoxicity

M. Rogge[1], **G. Fischer**[1], **U. Pindur**[1,*], and **D. Schollmeyer**[2]

[1] Institut für Pharmazie, Universität Mainz, D-55099 Mainz, Germany
[2] Institut für Organische Chemie, Universität Mainz, D-55099 Mainz, Germany

Summary. The cycloadducts **3**, **5**, and **7**, readily available from methoxy-substituted 3-vinylindoles **1** and **2**, were dehydrogenated with *DDQ* to the coplanar [*a*]-anellated carbazoles **4**, **6**, and **8**. Compound **4a**, also characterized by X-ray structural analysis, shows significant cytotoxicity against K562 und RXF393 human tumor cell lines.

Keywords. 3-Vinylindoles; Carbazoles; *Diels-Alder* reactions; Cytotoxicity.

[*a*]-Anellierte Carbazole mit Antitumoraktivität: Synthese und Cytotoxizität

Zusammenfassung. Die aus den methoxysubstituierten 3-Vinylindolen **1** und **2** zugänglichen Cycloaddukte **3**, **5** und **7** lassen sich mit *DDQ* zu den coplanar-anellierten Carbazolen **4**, **6** und **8** dehydrieren. Verbindung **4a**, die auch durch eine Röntgenstrukturanalyse charakterisiert wurde, zeigt signifikante Cytotoxizität gegen Humantumor-Zellinien (K562 und RXF93).

Introduction

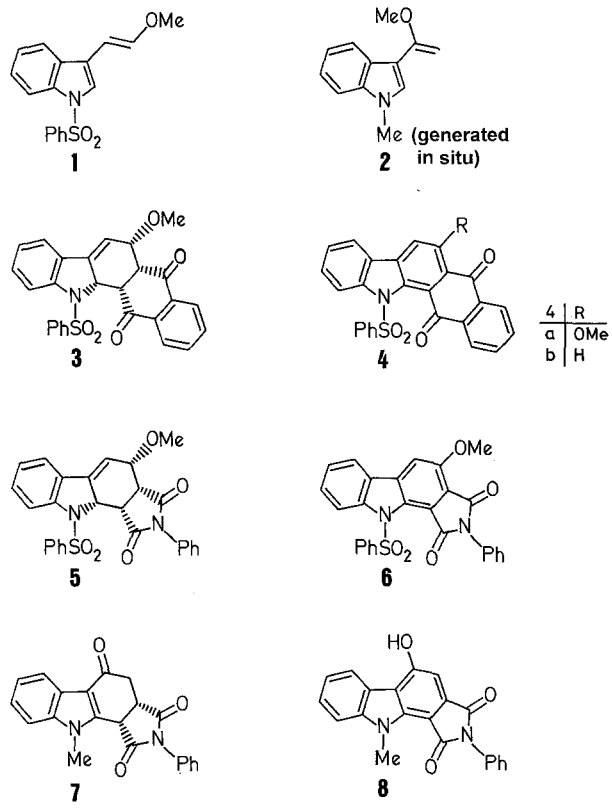
Due to their antitumor activity, coplanar anellated carbazoles are of general interest as intermediates in the development of new chemotherapeutic drugs [1–3]. In this field, derivatives of the pyrido [4,3-*b*] carbazole alkaloid ellipticine, which intercalates with DNA and inhibites topoisomerase II, are used clinically to stop the growth of several human tumors [1]. The chemistry of carbazoles and carbazole alkaloids has been extensively reviewed by several groups [1, 2, 4–6], and highly selective strategies have been reported for the synthesis of this class of compounds [7–12]. One principle of these concepts for obtaining selectively functionalized carbazoles is the *Diels-Alder* reaction of vinylindoles with appropriate carbodienophiles as a key step [8–12]. In continuation of our studies in the field of pericyclic reactions with indole derivatives [11–14], we report on three dehydrogenation reactions of the racemic carbazoles **3**, **5** and **7**, readily available from *Diels-Alder* reactions of 3-vinylindoles **1** and **2** with 1,4-naphthoquinone or N-phenylmaleimide. The cytotoxicity of the dehydrogenated [*a*]-anellated carbazoles was studied on the basis of growth inhibition tests with some human tumor cell lines.

Results and Discussion

Synthetic Aspects

We have already described the *Diels-Alder* reactions of β-methoxy-substituted 3-vinylindole (**1**) with 1,4-naphthoquinone and N-phenylmaleimide to generate the tetrahydro-carbazoles **3** and **5** [11, 12] as well as the *Diels-Alder* trapping reaction of *in situ* generated 3-vinylindole (**2**) with N-phenylmaleimide to produce the trioxo-functionalized pyrrolo[*a*]annellated carbazole **7** [13]. After testing several dehydrogenation catalysts, we found that 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (*DDQ*) seems to be the most appropriate reagent for the direct synthesis of fully aromatized carbazoles from partially hydrogenated carbazoles. In toluene (80–90 °C; 30 min) the dehydrogenation product **4a** is formed as the main component.

At a higher reaction temperature and with a longer reaction time (111 °C, 12 h), methanol elimination occurred additionally, giving rise to compound **4b**. In an analogous way, the hydrocarbazoles **5** and **7** were oxidized with *DDQ* in boiling toluene, yielding exclusively 14π carbazoles **6** and **8**, respectively.



Structural Aspects

The constitutions of the new carbazoles **4**, **6** and **8** were unambiguously determined by high resolution ^{1}H NMR spectroscopy. In the case of carbazole **4a**, an X-ray crystal structure analysis was performed (Fig. 1). This provided valuable geometrical

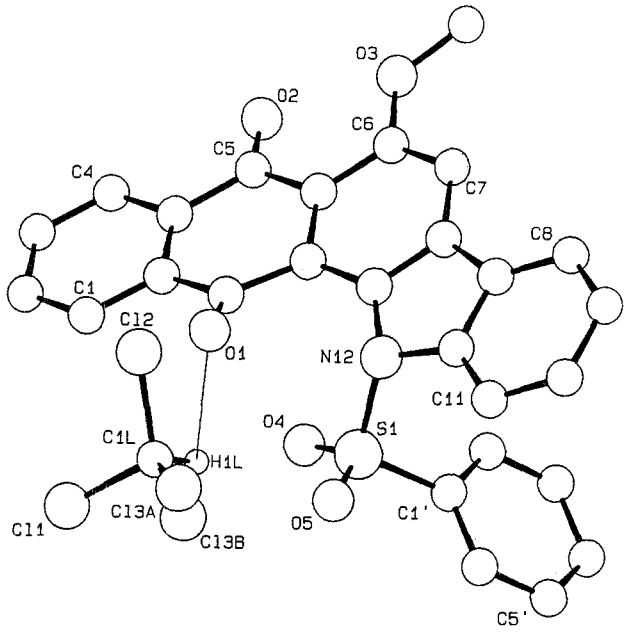


Fig. 1. PLUTO plot [17] of the molecular structure of **4a** in the solid state. The hydrogen bond is indicated by a thin line

information for our molecular modelling studies of carbazole-DNA intercalation complexes [15]. There is experimental evidence that the formation of carbazole-DNA complexes is the most biologically relevant principle of the cytotoxicity of this class of heterocyclic compounds [16].

In the crystal state, **4a** revealed a slightly pyramidalized carbazole nitrogen (angle sum around N12 = 342.8°) which is probably due to sterically demanding groups surrounding the nitrogen center. The anthraquinone constituent structure is twisted around its long axis. Compound **4a** crystallizes with one $CHCl_3$ molecule bound by a hydrogen bond (O1—C1L = 3.145(5) Å, see Fig. 1). One chlorine atom is disordered as indicated by the labelling 13A/B in Fig. 1.

Biological Assay, Cytotoxicity

The coplanar carbazoles **4**, **6** and **8** were submitted to *in vitro* cytotoxicity tests with four human tumor cell lines as a primary screening. From these investigations using the MTT assay [20], compound **4a** shows significant cell growth inhibition (Table 1, $IC_{50} = 25\ \mu M$ und $30\ \mu M$) of chronic myeloid leukemia tumor and kidney tumor cell lines (K562, RXF393). Thus, further structural synthetic variations of the "lead" **4a** will be performed, and more comprehensive biological studies are under investigation.

Experimental

^1H NMR spectra were recorded at 200 and 400 MHz with Bruker AC 200 and 400 spectrometers. The EI (70 eV) mass spectra were recorded on a Varian MAT 7 spectrometer. Elemental analysis were performed using a Carlo Erba Strumentazione 1106 apparatus. Melting points were measured with an

Table 1. Cytotoxicity MTT assay [20] of carbazole **4a**; human tumor cell lines: K562 (chronic myeloid leukemia), RXF393 (kidney tumor), HCT116 (colon cancer), SKmel 28 (malignant melanoma)

Tumor cell line	IC_{50}[a]	LC_{50}[a]
K562	25	–
RXF393	30	85
HCT116	>100	>100
SKmel 28	50	60–78

[a] IC_{50}: growth inhibitory concentration of carbazole **4a** (μM) needed to reduce cell number by 50%; LC_{50}: lethal concentration of **4a** (μM) needed to kill 50% of cells

Electrothermal 8200 instrument. Flash chromatograpy was performed on Merck 60 silica gel (particle size: 0.040–0.063 mm). The petroleum ether used had the boiling range of 40–60 °C.

General Procedure for Dehydrogenation of Carbazoles **3**, **5**, *and* **7**

The carbazoles **3** [12], **5** [12], and **7** [13] were dissolved in 20–50 ml toluene. Then, 2,3-dichloro-4,6-dicyano-1,4-benzoquinone (*DDQ*) was added and the whole mixture was heated from 80 to 111 °C. For more detailed reaction conditions, see below. After cooling the mixture, the volume of the solution was doubled and extracted three times with 20 ml portions of a saturated aqueous solution of $NaHCO_3$. Then the organic layer was washed with water until neutral, dried over $MgSO_4$, and concentrated in vacuum to a volume of 5 ml. After cooling the solution for 12 h at 2–8 °C, the precipitate was separated, purified by flash chromatography and crystallized.

6-Methoxy-12-(phenylsulfonyl)12H-naphtho[2,3-a]carbazole-5,13-dione (**4a**)

From 80 mg (0.17 mmol) **3** and 100 mg (0.44 mmol) *DDQ*; reaction conditions: 20 ml toluene, 3 h, 80–90 °C; yield: 41 mg (51%); m.p.: 255 °C (toluene). ^{1}H NMR (200 MHz, *DMSO*-d_6): $\delta = 4.07$ (s, 3H, OCH_3), 7.12–7.43 (m, 6H, H-aromat.), 7.57 (pseudo-t, $^3J = 7.75$ Hz, 1H, C8- or C9-H), 8.05 (mc, 7H, H-aromat.) ppm; EI-MS: m/z (%) = 467 (30) [$M^{+\cdot}$], 326 (100), 141 (2); $C_{27}H_{17}NO_5S$ (467.5); calcd.: C 69.37, H 3.67, N 3.00, S 6.86; found: C 69.40, H 3.86, N 3.11, S 6.85.

12-(Phenylsulfonyl)-12H-naphtho[2,3-a]carbazole-5,13-dione (**4b**)

From 100 mg (0.21 mmol) **3** and 100 mg (0.44 mmol) *DDQ*; reaction conditions: 50 ml toluene, 12 h, 111 °C; work up: flash chromatography (petroleum ether/ethyl acetate 1:1); yield: 35 mg (39%); m.p.: 340 °C (toluene). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.17$ (pseudo-t, 2H, H-aromat.), 7.34 (mc, 4H, H-aromat.), 7.45 (mc, 1H, H-aromat.), 7.80 (mc, 3H, H-aromat.), 8.02 (d, $^3J = 8.38$ Hz, 1H, H-aromat.), 8.15 (pseudo-t, $^3J = 8.38$ Hz, 1H, H-aromat.), 8.31 (mc, 2H, H-aromat.), 8.44 (d, $^3J = 7.94$ Hz, 1H, H-aromat.) ppm; EI-MS: m/z (%) = 437 (18) [$M^{+\cdot}$], 296 (100), 141 (13), 104 (10), 78 (11); $C_{26}H_{15}NO_4S$ (437.5); calcd.: C 71.38, H 3.46, N 3.20, S 7.33; found: C 71.27, H 3.31, N 3.02, S 7.18.

4-Methoxy-2-phenyl-10-(phenylsulfonyl)-1,2,3,10-tetrahydropyrrolo[3,4-a]carbazole-1,3-dione (**6**)

From 50 mg (0.10 mmol) **5** and 100 mg (0.44 mmol) *DDQ*; reaction conditions: 20 ml toluene, 30 min, 110 °C; yield: 34 mg (69%); m.p.: 252–253 °C (methanol). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.14$ (s, 3H, OCH_3), 7.35 (mc, 4H, H-aromat.), 7.48 (mc, 6H, H-aromat.), 7.62 (s, 1H, C5-H), 7.70 (dd, $^3J = 8.43$ Hz, $^4J = 0.93$ Hz, 2H, H-aromat.), 7.81 (d, $^3J = 7.62$ Hz, 1H, C4″-H), 7.95 (d, $^3J = 8.29$ Hz, 1H, C4′-H) ppm; EI-MS: *m/z* (%) = 483 (100) [$M^{+\cdot}$], 256 (35), 123 (12); $C_{27}H_{18}N_2O_5S$ (482.5); calcd.: C 67.21, H 3.76, N 5.81; found: C 67.60, H 3.84, N 5.52.

4-Hydroxy-10-methyl-2-phenyl-1,2,3,10-tetrahydropyrrolo[3,4-a]carbazole-1,3-dione (**8**)

From 400 mg (1.16 mmol) **7** and 300 mg (1.3 mmol) *DDQ*; reaction conditions: 30 ml toluene, 1 h, 110 °C; yield: 377 mg (95%); m.p.: 313 °C (petrol ether/ethyl acetate). ^{1}H NMR (200 MHz, *DMSO*-d_6): $\delta = 4.12$ (broad s, 1H, OH, exchangeable with D_2O), 4.33 (s, 3H, N-Me), 7.09 (s, 1H, H-C4-aromat.), 7.27–7.35 (mc, 1H, H-aromat.), 7.39–7.62 (m, 7H, H-aromat.), 8.29 (d, $^3J = 7.73$ Hz, 1H, H-aromat.) ppm; EI-MS: *m/z* (%) = 342 (100) [$M^{+\cdot}$], 297 (22), 250 (12), 195 (11), 166 (10), 139 (12); $C_{21}H_{14}N_2O_3$ (342.4); calcd.: C 73.68, H 4.12, N 8.18; found: C 73.34, H 4.17, N 8.08.

Crystal Structure Determination of **4a** [21]

$C_{27}H_{17}NO_5S \cdot CHCl_3$, $M_r = 586.84\,g \cdot mol^{-1}$, triclinic, space group *P*1- (no.2), $a = 8.367(2)$ Å, $b = 12.2115(5)$ Å, $c = 13.4959(7)$ Å, $\alpha = 69.309(4)°$, $\beta = 86.643(8)°$, $\gamma = 88.209(5)°$, $V = 1287.6(3)$ Å^3, $Z = 2$, $D_c = 1.514\,g \cdot gm^{-3}$, $F(000) = 600$, $T = 298$ K, Enraf-Nonius CAD4, graphite monochromator, $\lambda(CuK_\alpha) = 1.5418$ Å, $\mu = 43.35\,cm^{-1}$, no absorption correction applied. Pale orange crystals, size $0.64 \times 0.16 \times 0.14\,mm^3$, scan type $\omega/2\theta$, 5356 measured reflections, $2.0° \leqslant \theta \leqslant 70.0°$, $-10 \leqslant h \leqslant 0$, $-15 \leqslant k \leqslant 15$, $-16 \leqslant l \leqslant 16$, 3 standard reflections every 4000 s, 10% variation, corrected with cubic spline approximation, *Lorentz*- and polarization correction, 4284 unique reflections ($R_{int} = 0.051$), 3297 observed reflections ($|F|/\sigma(F) > 4.0$). Structure solution: direct methods (SHELXS86 [18]), full matrix refinement (SHELX-76 [19]), 343 refined parameters, H atoms located from difference Fourier maps, all atoms except H anisotropic refined, final $R = 0.056$, no weighting applied, max(Δ/σ) 0.007, min/max height in final ΔF map 0.3509/−0.4162 eÅ^{-3}. Figure 1 shows the molecular structure and the atom numbering according to IUPAC.

Acknowlegements

We thank the *Deutsche Forschungsgemeinschaft*, Bonn, for financial support and Dr. *A. Burger*, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD (USA) for performing the biological assay.

References

[1] Gribble GW (1990) In: Brossi A (ed) The ellipticine alkaloid. In: The alkaloids, vol 39. Academic Press, New York London, pp 239–343
[2] Chakraborty DP, Roy S (1991) Carbazole alkaloids. In: Herz W, et al (eds) Fortschritte der Chemie Organischer Naturstoffe **57**: 71–162
[3] Kuckländer U, Pitzler H, Kuna K (1994) Arch Pharm (Weinheim) **327**: 137–142
[4] Moody CJ (1994) Synlett: 681–688
[5] Knölker H-J (1992) Synlett: 371–387
[6] Pindur U (1990) Chimia **44**: 406–411
[7] Knölker H-J, O'Sullivan N (1994) Tetrahedron **50**: 10893–10908
[8] Wirth T, Blechert S (1994) Synlett: 717–718

[9] Wiest O, Steckhan E (1993) Angew Chem **105**: 932–934
[10] Gürtler CF, Blechert S, Steckhan E (1994) Synlett: 141–142
[11] Pindur U (1995) Cycloaddition reactions with indole derivatives. In: Moody CJ (ed) Advances in nitrogen heterocycles, vol 1. JAI Press, Greenwich, pp 121–171
[12] Pindur U, Kim M-H, Rogge M, Massa W (1992) J Org Chem **57**: 910–915
[13] Pindur U, Rogge M, Rehn C, Massa W, Peschel B (1994) J Heterocycl Chem **31**: 981–988
[14] Rogge M (1994) Thesis, University of Mainz
[15] Dräger M, Haber M, Erfanian-Abdoust H, Pindur U, Sattler K (1993) Monatsh Chem **124**: 559–576. Sattler K (1994) Thesis, University of Mainz
[16] Pindur U, Haber M, Sattler K (1993) J Chem Educ **70**: 263–272
[17] Motherwell S (1978) PLUTO 78, Crystallographic Data Centre, University Chemical Laboratories, Cambridge, England; modified by: Clegg B, Anorganisch-Chemisches Institut der Universität, Göttingen, Germany; VAX version: Schollmeyer D, Institut für Organische Chemie, Universität Mainz, Germany
[18] Sheldrick GM (1986) SHELXS86. Program for the solution of crystal structure. University of Göttingen, Germany
[19] Sheldrick GM (1976) SHELX-76. Program for crystal structure refinement. University of Göttingen, Germany
[20] MTT assay (micro-titer plate tetrazolium cytotoxicity assay for evaluating relative cytotoxicity): Mosmann TJ (1983) Immunol Methods **65**: 55–59. Weinkauf RL, LaVoie EJ (1994) Bioorg Med Chem **2**: 781–786
[21] Further details of the crystal structure investigation are available on request from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information mbH, D-76344 Eggenstein-Leopoldshafen, on quoting the depository number CSD 401708 for **4a**, the names of the authors, and the journal citation

Received June 19, 1995. Accepted (revised) August 1, 1995